THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

Sensor-based Methods for an Improved Understanding of Biological Water Treatment

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Gothenburg, Sweden 2024

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Technical report no 2024:1
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Cover:
An illustration of possible applications for oxygen sensors and fluorescence measurement methods, developed, and evaluated in this project. (Top) detecting trends of labile organic matter throughout drinking water treatment plants. (Bottom) monitoring removal efficiencies in parallel filter beds.

Illustration: Aina McEvoy

Chalmers Reproservice
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ABSTRACT

Access to clean and safe drinking water is essential for a functioning society. To produce drinking water in Sweden, raw water is taken from a natural resource and treated at a drinking water treatment plant, before being distributed to consumers via a pipe network. One important role of drinking water treatment is to remove natural organic matter (NOM). Natural organic matter is a collective term for a variety of organic compounds present in all natural water sources. The amounts as well as composition and characteristics of NOM vary between water resources as well as between seasons. In general NOM does not itself impose a health risk but it can alter the taste, colour, and odour of water, and may cause complications during drinking water treatment and distribution. One cause of concern for drinking water providers is microbial regrowth in treated drinking water while it is being transported in the distribution network. When drinking water is biologically stable, the microbial quality of water leaving the treatment plant will not change as it travels to the consumer, when unstable there can be substantial bacterial regrowth in the distribution network, potentially including pathogens. Bacterial regrowth is prompted by the presence of biologically labile fractions of NOM that bacteria are able to utilise as nutrients. There is a need for inexpensive tools that help to monitor biologically labile NOM throughout drinking water treatment and assess the effects of specific treatment steps on biostability. This thesis focuses on two new monitoring methods for measuring the availability of biologically labile NOM fractions, based upon (1) rates of oxygen consumption by bacteria, and (2) the composition of fluorescent NOM. The approach taken was to evaluate if these two methods could track labile organic carbon through dilution series, and secondly use each method to compare treatment processes at full-scale drinking water treatment plants. Treatment steps involving biological processes were studied particularly closely, because these are usually effective at removing labile organic carbon. Both new methods demonstrated potential for assessing the efficiencies of different types of treatment steps for removing labile NOM, for comparing performances of parallel filter beds, as well as for detecting changes in treatment efficiencies over time.

Keywords: Drinking water treatment, labile organic carbon, oxygen sensors, fluorescence measurements.

Preface

This thesis is based on work conducted between March 2022 and February 2024. The project was financed by funding from the Swedish Research Council FORMAS (2021-01411), Åke och Greta Lissheds Stiftelse (2021-00162), Gustav Richters Stiftelse (2021-00668) and Chalmers ICT Area of Advance. This research was performed within the DRICKS drinking water research framework hosted at Chalmers University of Technology. In this thesis a submitted manuscript is included

McEvoy A., Paul C., Modin O., Mohammadi A. S., McKelvey T., Murphy K., Two novel methods for studying the production and removal of labile organic carbon in water, *Manuscript* – Under review (submitted 15th December 2023).

Acknowledgements

I would like to acknowledge my main supervisor Kate for her guidance and support throughout the project-thank you, my co-supervisors Oskar, Catherine, Urban for their insightful input on questions that came up during the project. I also want to acknowledge Amir for all the help and input. I want to thank all collaborating drinking water treatment plants for their keen interest and help with sampling during this project, Kristina Holm, Olof Bergstedt and Anette Hansen (Göteborg Kretslopp och Vatten), Ämma Pettersson (Nodra AB), Erika Ljunggren and Johanna Hilding (Trollhättan Energi AB),), Daniel Hellström (Norrvatten), Caroline Schleich (VIVAB) and Andrew Holmes, Sigrún Ólafsdóttir, Linn Pedersen (Kungälv drinking water treatment plant).

I would also like to thank my present and past colleagues at WET, for lunches, fikas, and discussions. Finally, I want to thank my family Hjalmar, Mom, Dad, Peter, Jonna, Spurtle for their support throughout the project, thankful to have you all in my life.

Abbreviations

AOC - Assimilable Organic Carbon

BAC - Biological Activated Carbon

BDOC - Biodegradable Dissolved Organic Carbon

C - Carbon

CDOM - Chromophoric Dissolved Organic Matter

DBP - Disinfection by-product

DOC - Dissolved Organic Carbon

DOM - Dissolved Organic Matter

DWTP - Drinking Water Treatment Plant

EEM - Excitation and Emission Matrice

FDOM - Fluorescent Dissolved Organic Matter

GAC - Granulated Activated Carbons

HCl - Hydrochloric acid

LOCr - Labile Oxygen Consumption rate

N - Nitrogen

NaAc - Sodium Acetate

NOM - Natural Organic Matter

NOX - *Spirillum* (NOX)

O_2 - Oxygen

OM - Organic Matter

P - Phosphorus

P17 - *Pseudomonas fluorescens* (P17)

PAC - Pulverised Activated Carbon

PARAFAC - Parallel Factor Analysis

PBS - Phosphate-Buffered Saline

POC - Particulate Organic Matter

PoP - Projection on PARAFAC

RDOC - Recalcitrant Dissolved Organic Carbon

TOC - Total Organic Carbon

UF - Ultra Filtration

UV - Ultraviolets

Contents

1. Introduction

The focus of this thesis lies in the evaluation and application of new tools for quantifying dissolved organic matter (DOM) in aquatic ecosystems and tracking its fate in water treatment. For drinking water producers, it is of great importance to reduce the risk of microbial regrowth occurring in treated water, as this can lead to undesirable effects on aesthetic qualities such as taste, odour, and colour. Additionally, this event may result in potential health risks for the consumer if wrong bacteria proliferate. In the coming sections different fractions of NOM and their role in drinking water treatment will be further explored. However, one NOM fraction emphasised is the biologically labile fraction. This labile fraction can facilitate bacterial regrowth and threaten the biological stability of water. Therefore, this it is of great importance to track and ensure that treatment steps adequately remove this fraction. If too much labile organic matter remains substantial bacterial regrowth can occur in the treated water once it has entered the distribution network. Therefore, the development and exploration of accessible, rapid, and relatively inexpensive methods for monitoring labile organic carbon is important in terms of facilitating regular analysis. The application of such methods could be beneficial for drinking water producers for monitoring the efficiency of treatment steps and to ensure good quality water. We investigated and developed two methods, one method based on oxygen consumption rates, Labile Oxygen Consumption rate (LOCr) and another based on fluorescence measurements, here referred to as Projection on PARAFAC (PoP).

1.1 Identification of research questions

As will be established further in the coming sections, tools are needed for drinking water treatment plants to ensure good quality water. Here, methods based on oxygen sensor and fluorescence measurement were evaluated for monitoring the biologically labile fraction of organic matter, and the following questions posed:

1) Can methods based on oxygen sensors and fluorescence measurements be employed for quantifying labile organic carbon in water samples?

2) What role could these methods have in the future monitoring of labile organic carbon to assess the efficiencies of water treatment steps?

1.2 Thesis outline

Two independent methods were explored for monitoring the labile organic carbon in water systems, one based on oxygen sensor measurements (LOCr) the other on fluorescence measurements (PoP),

In the background section different fractions of NOM and their properties will be described, thereafter a general overview of drinking water treatment will be given. Moreover, the role of NOM fractions in the treatment of drinking water will be discussed, particularly the importance of being able to track the labile organic carbon fraction. Following this, the theory of the two methods evaluated, LOCr and PoP, will be introduced and related to characteristics of NOM.

In the method section, an overview of the experimental design will be described. This will be followed by a brief description of the sampling campaigns and sampling procedure. Finally, the technical details of the methods, and data analyses will be described.

The investigation was divided into three phases, so too the result section of this thesis is divided in three parts. The first part evaluates the potential for both methods of tracking the biodegradable fraction of organic matter. In the second part of the results section, method development and optimisation of the LOCr method will be described. In the third part of the results section, results from full-scale drinking water treatment processes evaluating the applicability of LOCr and PoP methods will be shown.

This thesis demonstrates the approach taken in evaluating these new methods. It includes an explanation of the experimental design, highlights challenges that were encountered, and motivates the decisions that were taken. Finally, based on the experimental results and with considerations of methodological limitations, possible applications of these methods are proposed.

2. Background

2.1 Natural organic matter (NOM)

NOM is matter that can alter aspects of the water such as colour, taste, odour, as well as facilitate biological metabolism (Health Canada, 2019; Singer, 1999; Volk et al., 2002). NOM is a term including a wide range of compounds, it is found in many different sizes, structures, and has a variety of accompanying properties (Huguet et al., 2009; Matilainen et al., 2010; Minor et al., 2014; Thurman, 1985). Due to these differences, it is difficult for treatment plants to detect and remove them efficiently and selectively (Health Canada, 2019; Matilainen et al., 2011; Volk et al., 2002). Based on their properties, NOM can be divided into different categories (Figure 1).

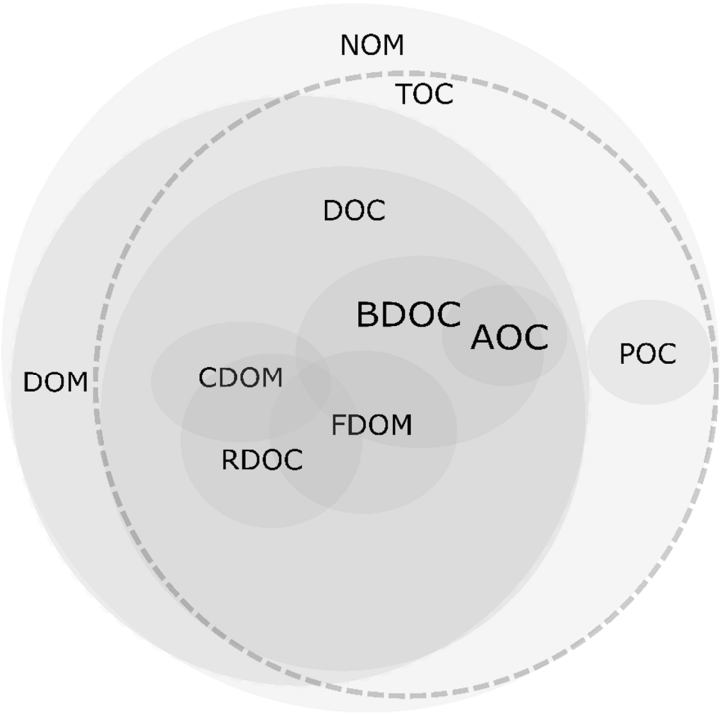

Figure 1: Natural organic matter (NOM) encompassing total organic carbon (TOC), which includes Particulate organic carbon (POC) and dissolved organic carbon/dissolved organic matter (DOC/DOM). Included in the DOC/DOM pool is biodegradable dissolved organic carbon (BDOC), chromophore dissolved organic matter (CDOM), recalcitrant dissolved organic carbon (RDOC), fluorescent dissolved organic matter (FDOM) and assimilable organic carbon (AOC).

Determined on the basis of size only, any NOM that passes through a 0.45 µm filter is defined as dissolved organic matter (DOM), whereby the carbon-containing fraction of this is called dissolved organic carbon (DOC). DOC makes up one of two fractions of total organic carbon (TOC), the other is made up of particulate organic matter (POC) (Health Canada, 2019; Thurman, 1985). Then there is coloured dissolved organic matter (CDOM), representing the organic matter that gives colour. These compounds absorb light at a wavelength range which results in lakes often

having a yellow or brown colour, one source of this CDOM stems from fallen leaves in the autumn (Blough et al., 1993; Hoge et al., 1993). Fluorescent dissolved organic matter (FDOM) in a similar way to CDOM can absorb light but in addition also has the property that it can re-emit light (Hoge et al., 1993). Biodegradable dissolved organic matter (BDOC) as well as the most biological labile fraction of BDOC, referred to as assimilable organic carbon (AOC), are fractions within DOC that can be metabolised by microbes. (Servais et al., 1995; Van Der Kooij et al., 1982). The fraction of DOC that is not biodegradable is referred to as recalcitrant dissolved organic carbon (RDOC) (Diem et al., 2013; Health Canada, 2019). The different types of NOM and their characteristics are compiled in Table 1.

Table 1: Classifications of natural organic matter and their characteristics and overlaps.

Natural organic matter (NOM)	Characteristics	Included in
Total Organic Carbon (TOC)	All organic carbon is encompassed by this category	NOM
Particulate Organic Carbon (POC)	Organic carbon that is excluded by a 0.45 µm filter	TOC
Dissolved Organic Carbon (DOC)	Organic carbon that passes through a 0.45 µm filter	TOC
Coloured Dissolved Organic Matter (CDOM)	The fraction of DOC that possesses the quality of absorbing light	DOM/DOC
Fluorescent Dissolved Organic Matter (FDOM)	The fraction of DOC and CDOM that can absorb then re-emit as fluorescence	DOM/DOC
Biodegradable Dissolved Organic Carbon (BDOC)	Relates to the fraction of DOC that can be metabolised by microbes. Partially overlapping with FDOM and CDOM	DOC/CDOM/FDOM
Recalcitrant Dissolved Organic Carbon (RDOC)	Relates to the fraction of DOC that cannot be biologically metabolised	DOC/CDOM/FDOM
Assimilable Organic Carbon (AOC)	The very labile fraction of BDOC, the small fraction of BDOC that is metabolised the easiest	TOC/BDOC

2.2 Drinking water treatment

According to the World Health Organisation and the European Commission, drinking water treatment plants (DWTPs) must be able to supply clean and safe drinking water (EC, 2020; WHO, 2017). The process from a raw water source to drinking water is a complex undertaking, and there are many variables as well as challenges that need to be addressed in order to achieve these goals.

The geographical location, as well as seasonal variations of abundance and character of NOM, affect the treatment needs of the incoming water (Ågren et al., 2008; Feng et al., 2023; Hessen et al., 1997; Zhang et al., 2021). Depending on the water source used for drinking water, the challenges vary. For instance, surface water such as lakes and rivers are more exposed to pollution particularly stemming from ships and run-off from roads, agriculture, and industries. Additionally, surface water may experience more seasonal variable pollution compared to ground water. Some examples of these variations are hazardous algal blooms during warmer summer months and seasonal lake turn-overs during spring and autumn (De Boer et al., 2019; Kraemer et al., 2015; MacIntyre et al., 1999; Pers et al., 2001; Sharp et al., 2006). All of the drinking water treatment plants (DWTPs) involved in this project, use surface water as their primary raw water source; the focus of this thesis therefore lies with surface water resources.

DWTPs treat the incoming raw water in multiple steps, and upon completing this treatment, it is crucial to maintain the quality of the treated water, all the way to the consumer's tap. After the water leaves the treatment plant, it moves into the distribution network and is often stored in reservoirs before delivery to the consumer. Multiple risks must be considered to ensure the quality of the treated water is preserved (Figure 2). All stages from water source to consumer must be addressed, to achieve the goal of safe drinking water. The focus of this project includes water treated at DWTPs.

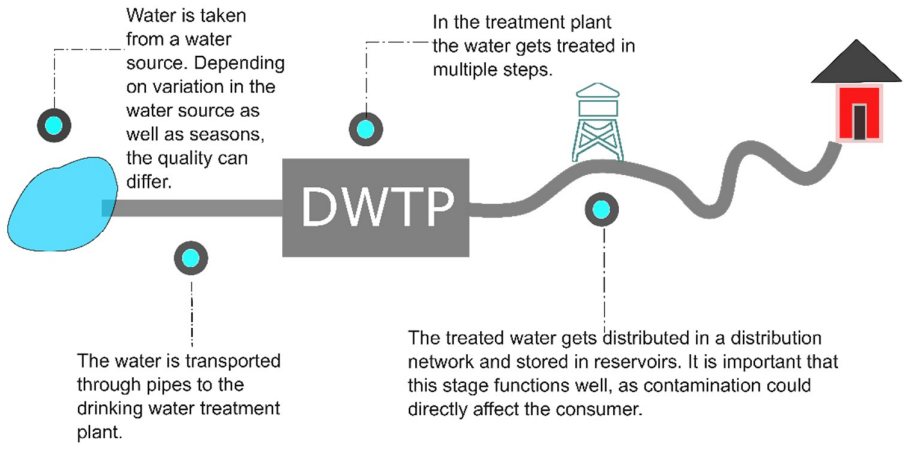

Figure 2: Illustration of the pathway taken by drinking water from a raw water source to the consumers tap, including water treatment, distribution through a pipe network, storage in water reservoirs and transportation to the tap. Highlighted are some water safety challenges along the way. The scope of this thesis will focus on the drinking water treatment plant (DWTP).

Drinking water treatment is an ever-evolving field. New guidelines regarding micropollutants (MPs) and environmental impact of treatment processes, including management of sludge and waste products, are all contributing factors to the need for optimisation (Bukhary et al., 2020; EC, 2020; Hofs et al., 2022; Nguyen et al., 2022; Tröger et al., 2018; WHO, 2017). Grasping this complexity requires a greater understanding of different treatment processes of drinking water.

2.3 Drinking water treatment and NOM

Which treatment processes are included, and to some extent the order in which these processes are applied, differ between DWTPs. For surface water, treatments often include coagulation, flocculation, sedimentation, filtration, and disinfection (De Boer et al., 2019). In Figure 3 and Table 2, several conventional drinking water treatment steps, encountered in DWTPs involved in this study, are displayed, and mentioned. In the coming paragraphs the effects of NOM on different treatments and of treatments on NOM itself will be discussed.

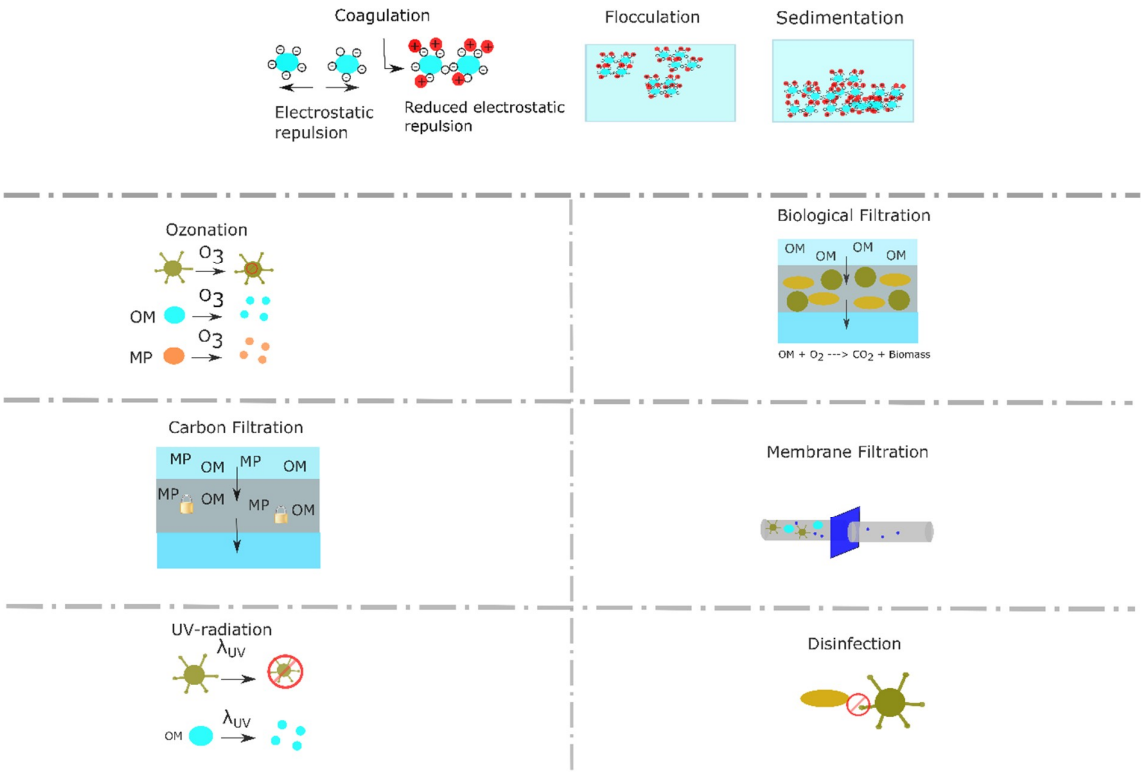

Figure 3: Conventional drinking water treatment processes encountered at DWTPs in this study, also depicted are how the treatments remove or transform matter.

Coagulation/Flocculation/Sedimentation

Coagulation removes NOM, which also reduces the turbidity of the water. Particles of NOM found in raw water sources often have a negatively charged surface and as a result experience repelling forces when approaching each other. The coagulant, which is often positively charged adsorbs to the negatively charged surfaces of particles, making them heavier. At the same time the positively charged coagulant neutralises the surface charges of NOM. The neutralised surface charge reduces the electrostatic repulsion and allow particles to come closer together (Jiang, 2015; Matilainen et al., 2010; Sillanpää et al., 2018; Sun et al., 2019; Bing-zhi et al., 2007; De Boer et al., 2019). Flocculation and sedimentation, commonly follow coagulation. Flocculation involves a slow mechanical turning that encourage coagulated particles to collide and form flocs of increasing size, this facilitates the removal of otherwise suspended NOM easier. Sedimentation is the process of larger particles sinking to the bottom as a sludge (De Boer et al., 2019; Matilainen et al., 2010).

Ozonation

Ozonation is a strong oxidising agent, which changes the composition of NOM and MPs, additionally ozonation also has the property that it deactivates bacteria. Ozonation is used for degradation of MPs, NOM and microorganisms, it can reduce unwanted odour, colour and taste. (Matilainen et al., 2006; Zhang et al., 2015) However, NOM and MPs are not entirely removed but rather transformed. Studies have shown that ozonation results in a decrease in high molecular weight fractions of NOM and an increase in low molecular weight fractions of NOM (A. Matilainen et al., 2006). Additionally, ozonation has been found to change structures of MPs to ozonation transformation products. The toxicities of these transformation products are often not as well explored as for the original MPs (Betsholtz et al., 2022; Huber et al., 2003; Soltermann et al., 2016).

Biological filters

Biological filters or also called biofilters include biodegradation processes. In these filters water passes through a filter media covered in biofilms. In addition to NOM removal via a physical barrier, the bacteria in the biofilms surrounding the granules (filter media) consume some of the labile organic matter fraction of NOM. Biological filters encompass different types of granules that facilitate biofilm formation on their surfaces such as sand or biological activated carbon (BAC). The latter combines adsorption properties of activated carbon with biodegradation in the biofilms (Abu Hasan et al., 2020; Morrisett et al., 2007; Visscher, 1990). Environmental factors, such as temperature, incoming water quality and aeration can alter the microbial community of the filter and in turn the removal mechanism and efficiency can differ (Bai et al., 2022).

Carbon filter

Carbon filtration commonly utilise particles of Granulated Activated Carbon (0.3-3 mm, GAC) or Pulverised Active Carbon (<0.05mm, PAC) (De Boer et al., 2019). With GAC water percolates through the GAC in a fixed filter bed, whereas PAC is injected into the flow. Activated carbon is used to remove MPs and NOM, by encouraging these to adsorb onto the surfaces of the activated carbon particles (De Boer et al., 2019; Karanfil, 2006). The removal efficiency depends on the adsorption properties of MPs and on the surface area and pore size of the activated carbon. MP removal can be impaired, by competition for adsorption sites with NOM and other substances. Particularly high molecular weight NOM fractions have a high sorption affinity to activated carbon (Abe et al., 1980; Day et al., 1994; Marais et al., 2018; Newcombe, 1994; Sillanpää et al., 2015). In general, the adsorption quality of the carbon filter diminishes as activated carbon ages

and adsorption sites become occupied (saturated). Carbon filters must therefore be periodically renewed or recharged through incineration, in order to restore their adsorption qualities (Larasati et al., 2021).

Membrane filters

Membrane filters have different sized pores and are categorised, in decreasing pore size as; microfilter (0.1-5 μm), ultrafilter (0.01-0.1 μm) and nanofilter (0.001-0.01 μm) (Koyuncu et al., 2015; Xu et al., 2022). With a range of pore sizes, the removal capacity of matter with different sizes also differs. Microfilters can remove bacteria, whereas ultrafilters additionally can remove viruses and proteins, nanofilters have pore sizes small enough to also remove ions. For smaller pore sizes the water needs to be pushed through with greater pressure, which is energetically costly (De Boer et al., 2019; Koyuncu et al., 2015; Xu et al., 2022). Complications with membrane filters include fouling of filters. One reason for fouling is NOM remaining clogged in the filter, even after back-washing. The hydrophobic fractions NOM have been found to cause the greatest decline in flux, meaning clogging of membrane filters, this is believed to be due to interactions between the matter and filter walls (Bing-zhi et al., 2007; Fan et al., 2001). Membrane fouling increases the ageing of the membrane and the maintenance associated with the treatment (Amy, 2008; Chew et al., 2016).

Chlorination

Towards the end of the treatment a disinfectant, such as different forms of chlorination (chlorine and chloramine) are added to the water. These disinfectants deactivate bacteria present in the water, preventing bacterial regrowth in distribution network (De Boer et al., 2019; Muntaha, 2021). Although the chlorine concentration decreases with distance from the DWTPs, the lasting effect of disinfection is important since the microbial quality of the treated water is maintained for longer as the water gets distributed. However, disinfectants in combination with residual NOM, particularly humic NOM fractions can form disinfection by-products (DBPs). During the 1970s it was found that DBPs cause some negative health effects (Bellar et al., 1974; X. F. Li et al., 2018; Muntaha, 2021; Rook, 1974). Other than NOM, nitrogen, and halogens, such as bromide also act as precursors for DBPs formation (Bond et al., 2012; X. F. Li et al., 2018; Singer, 1999).

UV radiation

Another method used is UV radiation (250-270 nm). This treatment deactivates bacteria, viruses, and fungi. During UV radiation the UV light is absorbed by DNA and RNA, making them incapable of replicating. Unlike with chlorination there is no residual disinfectant remaining after treatment and hence no extended disinfection property in the distribution network (Bolton et al., n.d.). Moreover, UV radiation has also been found to fragment organic matter into to smaller, and more biological available fractions (Heringa et al., 2011; Lehtola et al., 2003).

Table 2: Summary of some advantages and disadvantages of common water treatment processes.

Treatment	Advantages	Disadvantages
Coagulation/Flocculation/Sedimentation	• Good removal of hydrophobic high molecular weight compounds • Removes bulk NOM enhancing the performance of sequential treatment steps	• Dosage difficult to predict • Produces sludge which must be disposed • Smaller NOM not as efficiently removed
Disinfectant	• Deactivate bacteria before they enter the distribution system • Reduces the risk for waterborne diseases by prohibiting bacterial growth	• Disinfectants can react with NOM and form DBPs, which have a negative health effect
Ozonation	• Removes (converts) MPs • Degrades larger aromatic OM • Can reduce competition between larger NOM fractions and MPs for adsorption sites on activated carbon	• Can form ozonation transformation products which have a negative health impact
Membrane filtration	• Removes small sized particles, usually at end stages of drinking water treatment • Can remove viruses	• Membrane fouling, caused by NOM clogging membranes
UV-radiation	• Disinfects the water, by damaging DNA and RNA	• Does not have a residual disinfectant property, can contribute to increased labile NOM
Biofiltration	• Removes labile NOM • Removes nutrients for bacterial regrowth in the distribution network	• Biodegradation efficiency is temperature dependant. • Provides little control over what bacteria are present
Carbon filtration	• Effectively remove MPs	• Saturation can occur quickly, reducing MP removal efficiency • Renewal of carbon filters has a positive carbon footprint

From the accounts of the treatments introduced in the previous paragraphs, it can be seen that solving one problem can sometimes be the beginning of a new one. Due to the complex composition and variations of NOM, removing all NOM fractions is a difficult undertaking. One treatment process may remove a specific fraction of NOM but may also convert this fraction to another fraction. Methods for tracking removal and/or production of NOM fractions throughout the treatment process, could provide an improved understanding of drinking water treatment.

2.3 Biostability of water

Water reaching the consumer should be of the same microbial quality as water leaving the DWTP. When water has a high level of "biostability", there will be no detectable regrowth of bacteria in the distribution network (Prest et al., 2016; Rittmann et al., 1984; Van Der Kooij, 2000). The NOM fractions BDOC and AOC primarily aid in the growth of bacteria. Bacterial regrowth can result in undesirable odour, taste, and colour and if pathogenic bacteria start growing it can impose a health risk (Prest et al., 2016). The small size of the labile organic fraction renders it troublesome to remove, but nonetheless it is very important to do. Microbial regrowth and biocorrosion of distribution pipes are examples of indirect problems, with delayed onset of challenges, caused by residual labile NOM remaining in the outgoing water (Lee et al., 1980; Liu et al., 2002; Prest et al., 2016; H. Sun et al., 2014).

Being able to monitor the performance of treatment steps for removing labile organic carbon, would be valuable for DWTPs. Moreover, the ability to detect when the removal capacity is reduced, would allow for implementation of actions to compensate for this. Examples of such actions are implementing longer filtration times and renewing of filters. The methods LOCr and PoP investigated in this project, build particularly on three NOM components: FDOM, BDOC and AOC. The concepts of the methods will be further introduced and explained in the coming *sections 2.4* and *2.5* as well as the *Method sections 3.3 and 3.4.*

To maintain biologically stable water, the cause for bacterial regrowth must be known. Bacterial regrowth occurs if conditions are favourable for bacteria. These conditions relate to temperature, flow, and presence of nutrients (Prest et al., 2016). The labile fractions of the NOM-pool, acting as a nutrient source, could be correlated to the bacterial regrowth potential in water. This is one application of the methods developed and evaluated in this project, LOCr and PoP.

Microbes found in drinking water present in the distribution network are chemoheterotrophic and aerobic (Van Der Kooij et al., 1982). Meaning that when they metabolise, they similarly to humans consume oxygen together with nutrients, primarily carbon (C) together with nitrogen (N), phosphorus (P) in their metabolic process (Van Der Kooij et al., 1982). One proposed way of evaluating the presence of nutrients would be to monitor oxygen consumption with oxygen sensors (LOCr). The other way is characterising the biodegradability of the NOM based on molecular structures, in terms of fluorescent properties (PoP).

2.4 Oxygen sensors

Oxygen sensors were evaluated as a detection method for monitoring the presence of rapidly biodegradable NOM. The sensors used were optical oxygen sensors vials SensorVial SV-PSt5-4mL® from PreSens® (PreSens - Precision Sensing GmbH). A sensor dish, SDR SensorDish®, is situated underneath the vials. This senor detects the fluorescence emission stemming from the luminescent dye, built into the vials. Oxygen acts as a quencher of the fluorescent signal, facilitating the monitoring of oxygen concentrations (Figure 4).

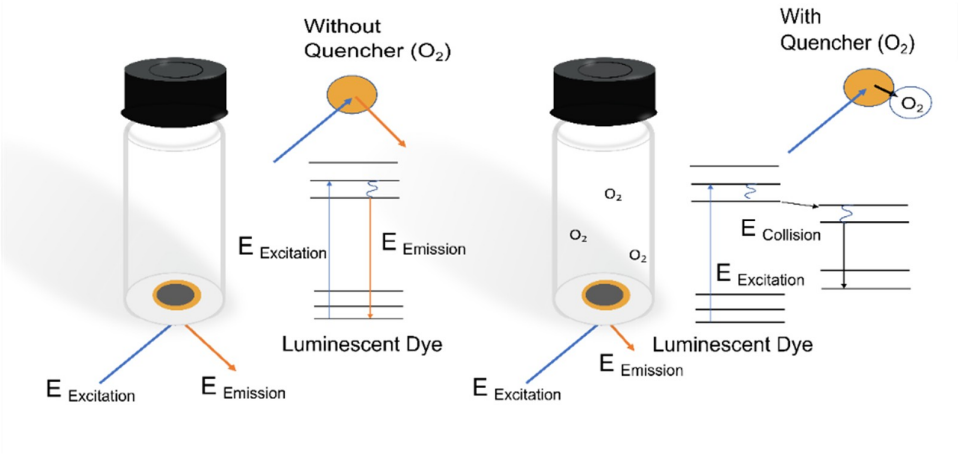

Figure 4: Schematic drawing of PreSens® sensor vials, illustrating the fluorescent dye and quenching mechanisms caused by oxygen. Left – the electrons in the dye get excited when hit with a photon and emits light as fluorescent light. Right – the electrons of the dye get excited when hit with a photon, if a collision with a O_2 molecule occurs, the energy gets quenched and does not emit light at the expected wavelength, due to energy conversion.

As mentioned in the previous section *(2.3),* heterotrophic aerobic bacteria consume oxygen when they metabolise. Therefore, an increased metabolism due to the presence of BDOC would result in an increased oxygen consumption. This theory is in fact the basis of a well-established method, referred to as *biological oxygen demand* (BOD). In BOD the oxygen consumption is measured at the beginning and end of an incubation period, typically 5 days. Based on the oxygen consumption, the cleanliness and safety of the water is assessed BOD (ISO 5815-1:2019, 2019; Jouanneau et al., 2014). This method is often used in wastewater treatment plants; however, the detection limit is insufficient for what is required for drinking water. The oxygen sensors method evaluated here, LOCr, follows a similar principal to BOD, in that oxygen consumption is measured over time. Often an initial steep decrease in oxygen concentration was a consistent feature early in the obtained oxygen consumption profiles. The oxygen consumption rate during this time-period was investigated and related to the abundance of the labile organic matter (Figure 5). Therefore, emphasis is put on the initial behaviour of the oxygen consumption profile since this part may best reflect the easily metabolized fraction of BDOC, and the parameter describing the initial maximum oxygen consumption rate is referred to as the Labile Oxygen Consumption rate (LOCr).

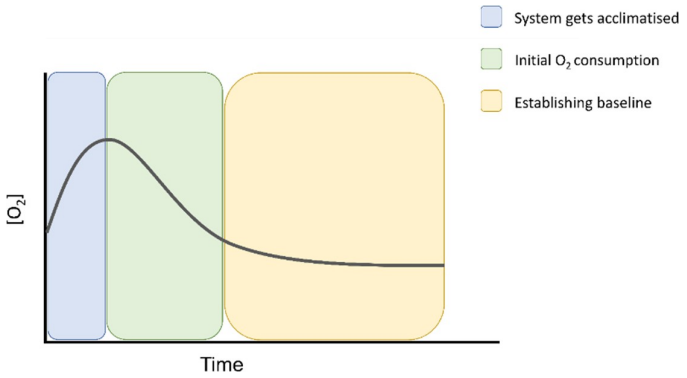

Figure 5: An oxygen consumption curve obtained during a three-day incubation period. Three regions of the cure are highlighted - Blue: The system is acclimatising, Green: Initial maximum oxygen consumption, and Yellow: Baseline conditions.

2.5 Fluorescence spectroscopy

With fluorescence spectroscopy one can detect fluorescent NOM (FDOM) in water samples. A schematic set-up of a fluorescence measurement is depicted in Figure 6. A light source passes through a monochromator. The monochromator allows a set narrow band of wavelengths from the original source to reach the sample. This is the wavelength, or the energy, that will be absorbed by the compound. Conventionally a monochromator is preceding a detector, and both are located at 90° to the light source. The 90° conformation is applied to minimise the risk of transmitted light from the light source reaching the detector. The emission monochromator scans different wavelengths; in this way the detector identifies at which wavelengths the emission/fluorescence occurs (Lakowicz, 2006).

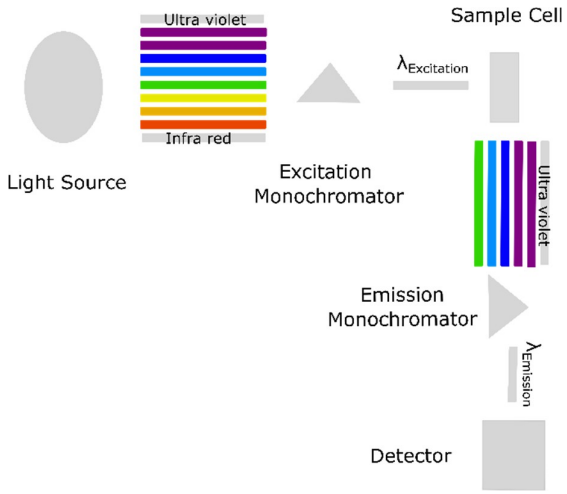

Figure 6: Fluorescence measurement. The light source emits light at multiple wavelengths, during a single measurement a monochromator selects the wavelength of the light that reaches the molecules in the test sample and cause electrons in these molecules to enter an excited state. The light emitted from the sample as a result of this excitation is detected at 90° to the sample to avoid transmitted light from the source. Using an emission monochromator, the wavelengths that are present in the emission are detected.

For a compound to be fluorescent, electrons of the compound must have the ability to absorb light and also re-emit light. Humic-like and protein-like NOM containing aromatic rings are such fluorophores. The fluorescent property is due to their conformation of electron sharing conjugated bonds (Lakowicz, 2006). When a photon hits a molecule, the electrons present in the ground state get excited to a higher energy level, corresponding to the energy of the photon used for the excitation. From here, the electron relaxes to the lowest energy level of the excited state. The electron then transits to the lowest energy level, and in that process emits light (fluorescence), at the wavelength corresponding to the energy gap between the states (Lakowicz, 2006). There is a slight shift in energy between the excitation (higher energy) and emission (lower energy). This shift is due to the small energy loss that occurs when the excited electron relaxes to the lower energy level of the excited state. The shift in energy is referred to as Stoke's shift (Figure 7). Structural variations result in different characteristic absorption and emission wavelengths.

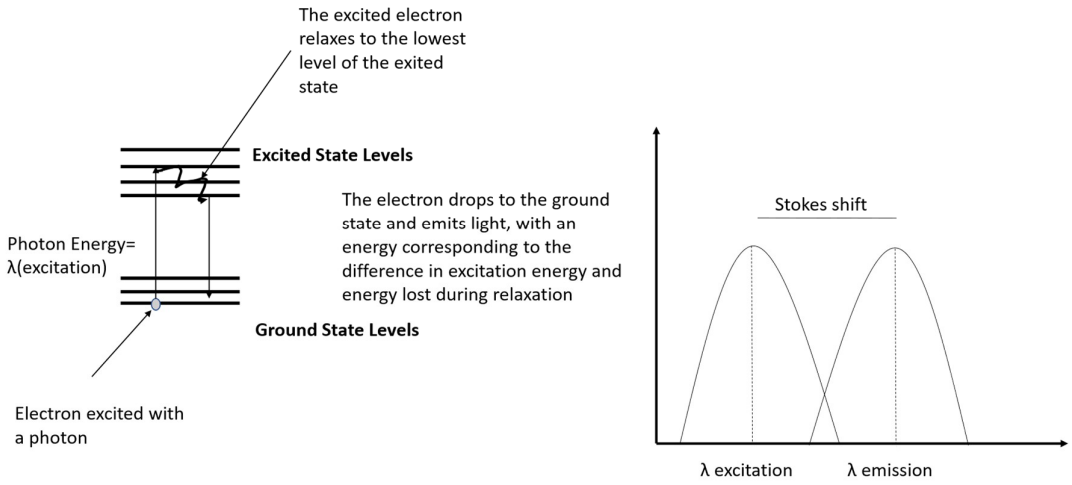

Figure 7: (Left) drawing of a Jablonski diagram, (Right) excitation and emission spectra separated by Stokes shift.

2.5.1 Excitation and emission matrices (EEMs)

Fluorescence spectroscopy is a sensitive method for identifying and quantifying analytes present in samples. This is possible due to the unique emission and adsorption properties of many known compounds (Lakowicz, 2006). However, natural water contains a wide range of compounds, and a complex matrix of NOM with different characteristics (Coble, 1996; Matilainen et al., 2011; Wünsch et al., 2019). Extracting absolute concentrations of analytes is, because of the complex composition, not possible. In order to obtain valuable information from water samples excitation and emission matrices (EEMs) of the samples are collected. For this, samples are irradiated with light of incremental monochromatic excitation wavelengths within a specified range. The emission at each wavelength is detected and a spectrum obtained. With incremental measurements, EEMs are created for the samples. These EEMs map out the complex composition of the water sample, almost like a DOM fingerprint of the sample (Baker, 2005; Coble, 1996;

Coble et al., 2014). Naturally EEMs contain a lot of data, to extract information, regarding the characteristics of DOM in the water samples, multivariate analysis is conducted on these EEMs.

2.6 Data analysis

2.6.1 Multivariate analysis

Compounds that are structurally similar, often behave similarly. Regions of signal positions, that have been linked to different FDOM fractions, can be extracted from an EEM using multivariate analysis (Baghoth et al., 2011; Moona et al., 2021; Murphy et al., 2018; Murphy et al., 2013, 2014; Wünsch et al., 2019). The obtained signals are processed with parallel factor analysis (PARAFAC). From this information on the FDOM composition of the sample can be obtained (Stedmon et al., 2005).

A model is created with PARAFAC, and reoccurring signal regions are identified (Murphy et al., 2013). The characteristics of some common peaks have been identified and categorised (Figure 8). It has been found that freshwater systems form ubiquitous EEMs when comparing samples from around the world (Murphy et al., 2018). A previous study by Moona et al. 2021 found that two PARAFAC components $\lambda_{Ex}/\lambda_{Em}$ 290nm/420nm and 280nm/340nm showed signal removal after biodegradation. These findings are the basis for exploring the use of an already existing PARAFAC model on new EMMs (Projection on PARAFAC, PoP), and evaluating this approach as an indicator of the biologically labile fraction of FDOM.

The PARAFAC model used in this thesis, Kungälv5 (Moona et al., 2021), identifies five FDOM components from an EEM; two biodegradable and three recalcitrant. For simplicity the nomenclature introduced in Table 3 will be used, the positions of these in an EEM are shown in Figure 8.

Table 3: Compiled are five FDOM components obtained with the Kungälv5 model (Moona et.al. 2022) and their corresponding excitation and emission wavelengths as well as their identified characteristics.

Components	$\lambda_{Ex}/\lambda_{Em}$ (nm)	Characteristics
H_A	340/445	Humic-like aromatic (Cuss et al., 2015; Philibert et al., 2022)
H_{BIO}	290/420	Humic-like biodegradable (Chen et al., 2015; Moona et al., 2021; Peleato et al., 2016)
H_{HMW}	360/510	Humic-like high molecular weight (Cuss et al., 2015; Philibert et al., 2022)
H_{LMW}	310/400	Humic-like low molecular weight (Philibert et al., 2022)
F_{Tryp}	280/340	Protein-like Tryptophan (Moona et al., 2021; Murphy et al., 2014)

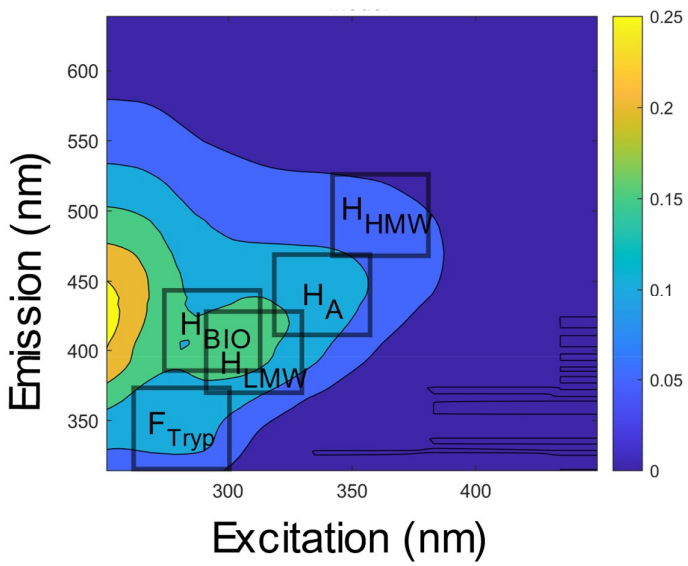

Figure 8: Peak positions for the five FDOM components obtained with the kungälv5 model; H_A, H_{BIO}, H_{HMW}, H_{LMW}, F_{Tryp} (Moona et.al 2022) in an excitation and emission matrix.

3. Methods

3.1 Experimental design

The experimental approaches for, oxygen sensors (LOCr) and fluorescence measurements (PoP), are depicted in Figure 9. From the figure, it is apparent that more developmental (trial and error) work was required for the LOCr method compared to PoP, which did not require method development. For LOCr the method development was at the first branch divided into two focus areas: *Pre-treatment* and *Inoculum.* Details about the evaluation of these areas are described in the *Methods* section *3.3.1, 3.3.2* as well as *Results and Discussion* section *4.2.*

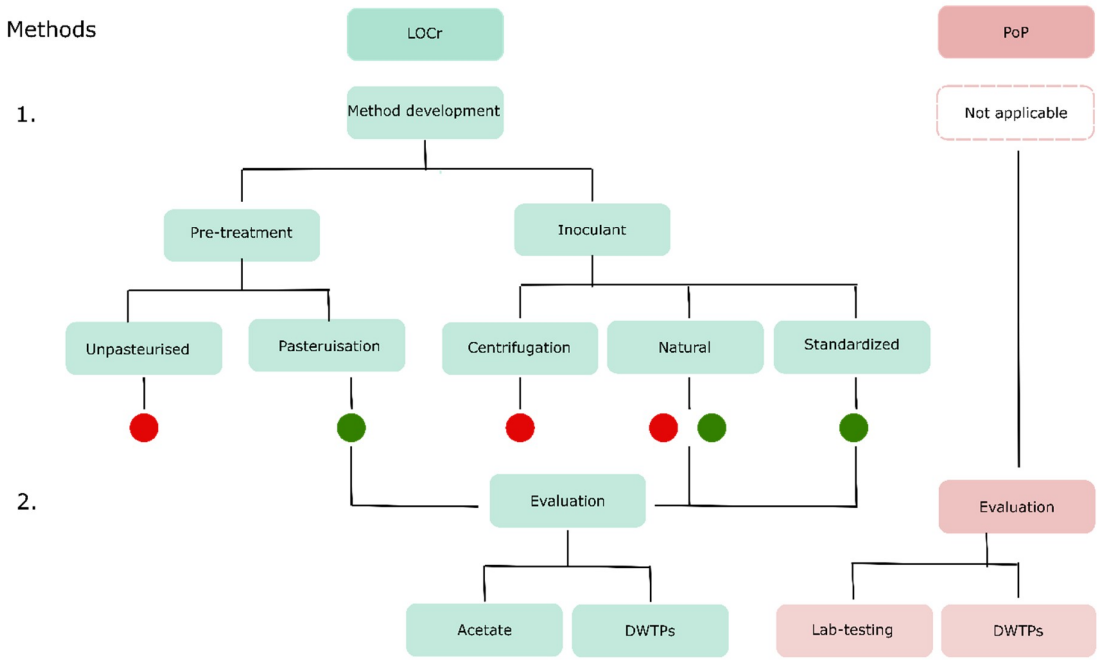

Figure 9: Schematic drawing of the experimental workflow. The oxygen sensor method involved a method development section, where red dots represent paths were considered unfruitful and green represents paths investigated further. Paths with red and green dots indicate the need for further refinement. As part of the evaluation for LOCr acetate dilution series and applications in the drinking water treatment plants (DWTPs) were investigated, for PoP the evaluation included Laboratory testing and applications in DWTPs.

Due to the differences in the methods, the evaluation processes also differed slightly. A short description of the approaches taken is given in the following chapters, and a summary is compiled in Table 4.

Table 4: Introducing the different stages of the evaluation process, including laboratory testing (Lab-testing) and application in Drinking water treatment plants (DWTPs) for LOCr and PoP. Each section including what was investigated, with what methods and for what purpose.

Lab-testing		
Acetate	LOCr	How does the LOCr method behave with a known labile organic carbon source?
Dilution	LOCr/PoP	How do the methods behave upon dilution?
Incubation test	PoP	Can a signal reduction be detected as a result of incubation?
DWTPs		
Biological processes	LOCr/ PoP	Are similar results seen in full-scale processes, as the incubation tests?
Raw to Outgoing	LOCr/ PoP	Can these methods track the removal of labile carbon throughout DWTPs?

3.1.1 Lab-testing

Initially, tests were conducted to see if the following theory holds true; that an increase in easily metabolised organic matter, results in an increased signal strength for both methods. For the LOCr method dilution series of sodium acetate (NaAc), acting as a labile carbon source, were used. Thereafter, it was investigated if a similar trend was seen for a dilution of natural water. For this a natural water source, treated waste-water, containing a more complex composition of DOM was used.

For PoP a standard carbon source of sodium acetate could not be used, as the model used in the method was built on EEMs of natural water. The correlation between fluorescence signal strength and dilution has previously been investigated and shown to behave conservatively if inner filter effects were corrected for (Kothawala et al., 2013). Instead, inoculated samples were measured before and after incubation (35°C), replicating the methodological procedure of LOCr. Through this, additional confirmation could be obtained regarding the understanding that biologically labile PoP component H_{BIO} was removed through biological degradation. Moreover, the experiments tested the hypothesis that the reduction of H_{BIO} reflected the removal caused by the metabolism observed with the LOCr method.

3.1.2 Drinking water treatment plants

Multiple sampling campaigns were conducted to evaluate the large-scale applicability of the methods. These campaigns were split into two categories described below. DWTPs involved in these campaigns are addressed with the following nomenclature: DWTP-K, DWTP-T, DWTP-G, DWTP-N.

Biofilter performance:
At DWTP-K raw water is, after aeration, treated with biofilters in four parallel beds. This built in replication provided a good set up for directly assessing the ability of LOCr and PoP to detect removal of organic carbon due to biological degradation. Samples were collected before and after biofiltration in the period of June 2022-March 2023.

DWTP campaign:
PoP and LOCr measurements were performed on samples collected at multiple DWTPs, in the period from September 2022 to August 2023. Samples were collected throughout the treatment plants, from incoming raw water to outgoing water, or water from the distribution network. The treatment steps at each plant differed slightly with respect to treatments used and/or sequential order. Table 5 lists the treatment steps for each DWTP. At all DWTPs, in this campaign, surface water was used as the raw water source.

The sampling of sequential treatment steps, using LOCr and PoP resulted in an overview of the removal or production of labile organic matter, at water treatment plants. It was further investigated if the PoP method could indicate where in the treatment process the selective removal of labile organic matter occurs.

Table 5: Compiled are treatment steps and order (1-6) for three different drinking water treatment plants (DWTPs) included in this study.

Point	1	2	3	4	5	6
DWTP-N (Autumn 2022)	Raw water	Flocculation/Flotation +Sand filter	Carbon filter	UV (pH adjustment and chlorination)	Outgoing	Distribution network
DWTP-T (Winter/Spring 2023)	Raw water	Coagulation/Sedimentation	Outgoing (after Rapid sand filter/Slow sand filter + UV)	Distribution network	-	-
DWTP-G (Summer 2023)	Raw water	Flocculation	Carbon filter	Outgoing water (Ultra filtration, Chlorination and pH adjustment)	-	-

Comparison with AOC methods:
Duplicate samples, for comparison purposes, were sent to a commercial laboratory for AOC analyses. In the commercial analysis of AOC the growth of *P. fluorescens* (P17) and/or *Spirillum* NOX are converted to AOC concentrations as acetate equivalents, using the protocol of Van Der Kooij and others, without the addition of nitrogen and phosphorous nutrients (Van Der Kooij et al., 1982, 1984).

3.2 Sampling

Samples were collected in baked amber glass bottles (550 °C, 6 h) and sealed with acid-washed lids (10 % HCl, 3 days), to minimise contamination of organic matter. During sampling the bottles were rinsed twice with the sample before being filled to the top, avoiding exposure to oxygen, which would promote the biological breakdown of NOM before analysis. Samples were stored cool prior to sample preparation and analysis, then filtered for fluorescence measurements (0.45 µm polyethersulfone-syringe filters) or pasteurised for oxygen sensor measurements. The sample pre-treatment was conducted as soon as possible after sampling, usually within 24 hours. Analysis was then nominally conducted within 48 hours of sampling.

3.3 Oxygen sensors (LOCr)

The LOCr method monitors oxygen consumption by bacteria over time. From this profile the oxygen consumption and oxygen consumption rate are extracted using an algorithm *(section 3.3.3).* The step-by-step process is illustrated in Figure 10.

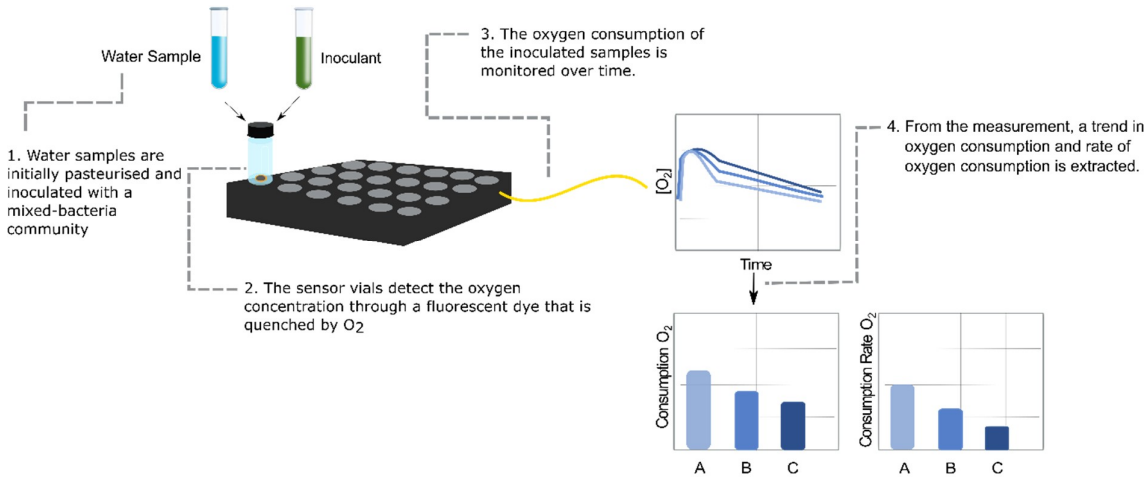

Figure 10: The analysis process of the oxygen sensor (LOCr) method. (1) Water samples are pasteurised and inoculated. (2) The oxygen concentration is measured over time with PreSens® oxygen sensor vials. (3) Measurements are logged every three minutes, and oxygen consumption profiles (Oxygen concentrations as functions of time) are obtained. (4) The initial maximum oxygen consumption and oxygen consumption rate is retrieved.

3.3.1 Inoculum

Two types of inoculants were evaluated in this project: a natural mixed community inoculant *(natural inoculant)* and a standardised mixed culture seed *(PolySeed®)*. Brief descriptions of these are given in the coming paragraphs and Table 6.

Natural Inoculum:
Water from a raw water source (Göta Älv) was incubated for 1 – 2 weeks at 35 °C and aeriated regularly. During inoculation it was important that the bacteria in the inoculum were accustomed to low nutrient environments, similar to drinking water samples. However, signal recovery was sometimes challenging. This was believed to be partly due to low concentration of viable bacteria, especially during winter months. Improvements were seen when spiking the sample with 1µg/L C as NaAc-Eq prior to adding the inoculum and starting the measurements.

PolySeed®:
PolySeed® (InterLab®) is a mixed culture bacteria seed, sold commercially as an inoculum seed for use when performing BOD measurements. The content of one capsule was hydrated in Phosphate Saline Buffer (PBS-buffer) for 1 h under magnetic stirring. Thereafter, left to settle for 15 min before it was dispensed as an inoculant.

Table 6: Compiled are descriptions of the *Natural inoculum* and Standardised *PolySeed®* inoculant used. Descriptions include sources and preparation steps.

Type of inoculant	Source	Preparation
Natural	Raw water source – Göta Älv	An amber glass bottle was filled to half with river water. Thereafter stored at 35°C for 1-2 weeks with regular aeration prior to inoculating samples.
PolySeed®	PolySeed® capsule	Capsules were hydrated according to manufacturer's instructions. 500 mL PBS-buffer in an autoclaved glass bottle. The mixture was stirred for 1 h and thereafter 15 min without stirring to allow sediment to settle.

3.3.2 Sample preparation and measurement

Aliquots of samples were dispensed into baked 40 mL amber glass bottles (rinsed twice with MilliQ water). The samples were sealed and pasteurised in an oven, at 70 °C for 45 min allowing for a warm-up time of 15 min (Lechevallier et al., 1993; Liu et al., 2002). Sample pasteurisation took place as soon as possible after sampling, when possible, within 24 h. The samples were acclimatised to 35 ° one hour prior to inoculation. This was done to reduce the time required for

the temperature to acclimatise once the measurement had started. Samples were inoculated according to Table 7; thereafter the 40 mL bottles containing the sample and inoculum, were carefully shaken to introduce oxygen into the sample before samples were dispensed into the sensor vials for measurement.

Table 7: Compiled are quantities of samples and inoculum for a total inoculated sample volume of 30 mL, used for measurements with the LOCr method.

Type of inoculant	Sample (mL)	Inoculant (mL)
Natural	29	1
PolySeed®	29.5	0.5

Sensor vials (SensorVial SV-PSt5-4mL®) from PreSens® (PreSens - Precision Sensing GmbH) were filled so that a convex surface was formed. This was to reduce the risk of hidden air-pockets affecting the measurement of dissolved oxygen concentration. Measurements were logged every 3 minutes over a 3-day period at 35 °C in an incubator.

Sodium acetate dilutions:
A stock solution of 5000 µg C/L as sodium acetate was prepared, then different concentrations were obtained through dilution with MilliQ. A fresh stock solution and dilution series was made for every new batch measurement.

3.3.3 Data analysis

The sensor output produced an oxygen consumption profile, showing oxygen concentration over time. An algorithm was developed in Matlab®, to identify when the initial rapid consumption of oxygen and thus metabolism of labile organic matter took place (Figure 5 in *section 2.4*).

A schematic drawing of the algorithm workflow is shown in Figure 11. The first derivative of the oxygen consumption profile was calculated, and the maximum negative peak was detected, representing the greatest change in slope i.e., *a drop,* giving the curve an S-shape. The duration of the drop is found by defining the tail end of the three-day measurement as a baseline. Thereafter, it was marked when the negative peak crosses the baseline. The magnitude as well as the duration of the drop could then be identified. The oxygen consumption is defined as the magnitude of the drop. Whereas the magnitude divided by the elapsed time between crossing the baseline is defined as the oxygen consumption rate.

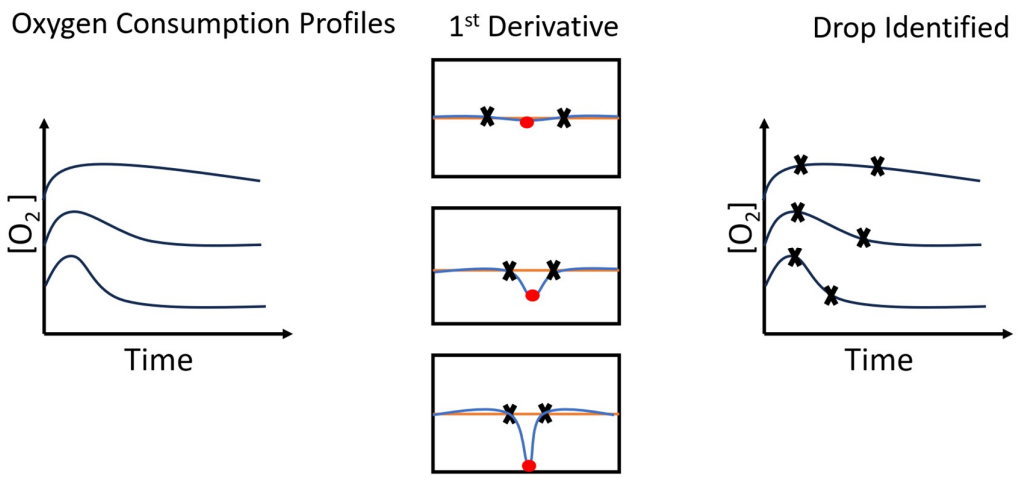

Figure 11: Drawing of how the algorithm identifies the duration when the maximum oxygen consumption takes place. In the left figure, the first derivative of the oxygen consumption profile is used to identify the largest negative peak. Middle figure, the duration of this drop is extracted by identifying when the baseline is reached again, relative to the tail end of the measurement. Left figure, lines depict increasing concentrations from top to bottom. Distance on the time axis between the X markers indicate the duration of maximum oxygen consumption identified by the algorithm.

For some oxygen sensor measurements single replicates or entire batches had to be manually eliminated, see Figure 12 for examples of eliminated replicates or measurement batches. Examples of eliminated data are based on interruptions in the S-shape, which may be due to air-bubbles present in the sample dissolving, once the dissolved oxygen was consumed. Other factors such as rapid changes in temperature resulted in clear interruptions of the oxygen consumption profile for the entire data set. Other reasons included small drops in the oxygen consumption profiles, which proved difficult for the algorithms signal recovery. In such cases the algorithm could not identify the duration of the drop, and therefore not extract the required oxygen consumption rate.

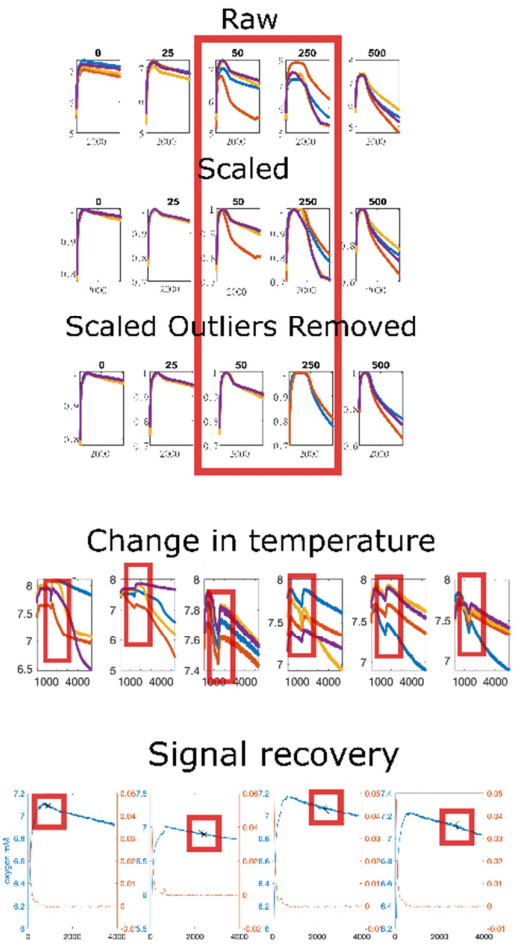

Figure 12: Examples of scenarios requiring removal of outliers. Top: single outliers identified by comparing with replicates, middle: unstable temperature affects all samples at the same time, bottom: entire dataset has too low signal.

3.4 Fluorescence spectroscopy (PoP)

3.4.1 Sample preparation and measurement

Samples were filtered (0.45 µm polyethersulfone syringe filters. A Horiba Aqualog® was used and emission and excitation of the samples were measured, a quartz crystal cuvette of 1 cm was used for the measurements spanning excitation wavelengths from 239 nm to 500 nm with 3 nm increments.

3.4.2 Data analysis

In short, an EEM of a sample was collected. The EEM was then projected on an *a priori* PARAFAC model Kungälv5 (Moona et al., 2021), five FDOM components extracted. The intensities of the regions were related to the relative abundance of the components. The methodological workflow and data analysis for PoP is illustrated in Figure 13.

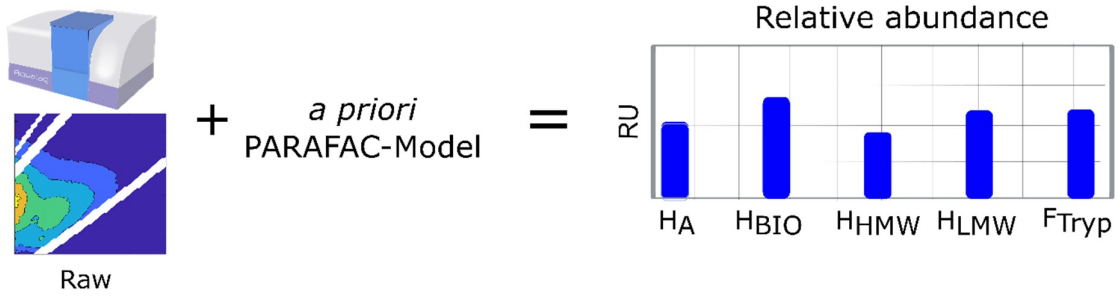

Figure 13: Schematic drawing of the PoP fluorescence measurements. An excitation and emission matrix is obtained from the fluorescence measurement and projected on an *a priori* PARAFAC model (Kungälv5). As an output, the signals in the EEMs are given as relative abundances of the five different FDOM components.

3.5 Dissolved organic carbon (DOC)

Filtered samples (0.45 µm polyethersulfone syringe filters) were dispensed in baked amber glass bottles (40 ml, 550 °C, 6h), in aliquots of three. A Shimadzu TOC-VCPH carbon analyser with auto-sampler TOC ASI-V was used. Analysis took place as soon as possible but no later than 48 h after sampling, samples were stored cool between sampling and analysis. If DOC analysis was not possible soon after sampling, the samples were acidified to pH<2, for preservation of NOM.

4. Results and discussion

4.1 Lab-testing

The Lab-testing section is divided in two parts, adapted to the differences of the methods. To recap, the LOCr method gives an account of labile carbon consumption rate by bacteria in terms of oxygen consumed. Whereas, PoP method yields an estimate of the labile organic matter content, in terms of relative abundance with regards to the total FDOM pool.

4.1.1 Oxygen sensors (LOCr)

Sodium Acetate:
The results of the acetate dilution series showed an increased oxygen consumption rate, as the concentration of NaAc increased (Figure 14). However, for high concentrations of NaAc the consumption gave the appearance of a Monod kinetic curve - suggesting that the method did track the growth of bacteria.

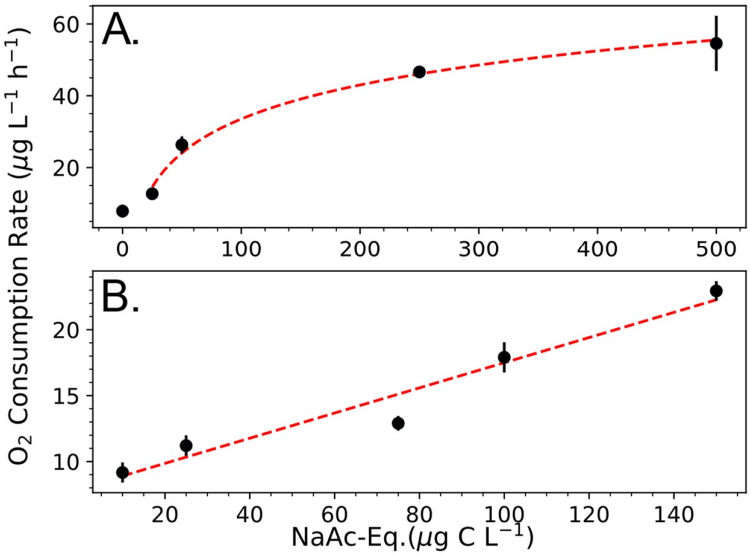

Figure 14: Oxygen consumption rates as functions of the labile organic carbon concentrations as NaAc equivalents, ranging from 0-500 µg C L^{-1} (A), and 10-150 µg C L^{-1} (B). Samples were inoculated with a natural inoculant. Error bars indicate standard errors of means.

Natural water:

A dilution series of secondary treated waste-water, containing a complex matrix of organic matter, in MilliQ showed a decrease in oxygen consumption rate together with an increased dilution factor Figure 15.

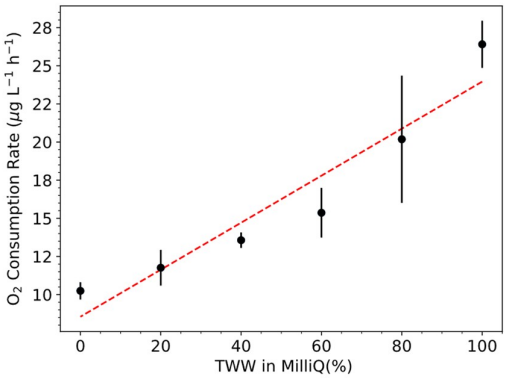

Figure 15: Oxygen consumption rates for dilution series of secondary treated wastewater in MilliQ. Sample was inoculated with PolySeed®. Error bars indicate standard errors of means.

Nutrients:

The effect of adding the nutrients nitrogen (N) and phosphorus (P) was also investigated. An excess of N and P with respect to a 100:10:1 (C:N:P) ratio was added (80:8:1), and resulted in a linear correlation between the presence of labile organic carbon and oxygen consumption rate (Figure 16). Adding an excess of N and P would provide an environment where carbon would be limiting, and perhaps would yield in a more accurate representation of the AOC content of the water. However, from a user applicability standpoint, it was argued that the regrowth potential in terms of substrate utilisation at natural nutrient compositions was important information to obtain. It was decided to omit additions of N and P in future measurements based on the following two accounts; 1) carbon, in natural water systems, is usually limiting in biomass production (Hammes et al., 2005; Emmanuelle I. Prest et al., 2016) and 2) so as not to alter the composition of the water if not necessary.

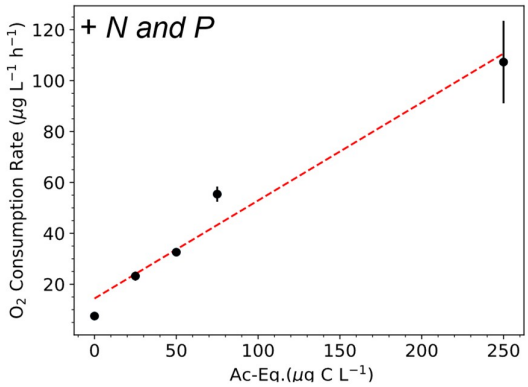

Figure 16: Oxygen consumption rate as a function of labile organic carbon source as NaAc equivalents with an 80:8:1 (C:N:P) ratio. Sample was inoculated with a natural inoculant. Error bars indicate standard errors of means.

In summary the following conclusions were made:

- *With an increase of labile organic carbon, the oxygen consumption increased. Over a concentration span of 0-500 µg C L^{-1} the oxygen consumption curve mimicked a growth curve.*
- *Additions of N and P were omitted in future measurements. It was argued that if the assumption that carbon being limiting does not hold true, this approach of omitting additions of N and P will perhaps best reflect regrowth potential rather than the AOC concentration.*

4.1.2 Fluorescence spectroscopy (PoP)

With fluorescence measurements the proof-of-concept approach was slightly different. This method identifies relative concentrations of FDOM fractions based on a PARAFAC model created form natural waters consisting of a complex composition of NOM. Thus, using known concentrations of a known compound could not be applied here. Incubation tests were conducted to confirm that the H_{BIO} signal extracted does in fact represents the biologically labile organic matter. These incubation tests were carried out in two stages.

Firstly, six replicates of samples from a raw water source (collected September 2022) were inoculated with a natural inoculant, applying the same methodology described for the oxygen sensor methods. Three replicates were filtered and measured, the other three were incubated at 35 °C for seven days, after incubation these three replicates were also filtered and measured. From this test the removal percentage for the five FDOM components could be extracted, it was found that two components (H_{BIO}, F_{Tryp}) showed removal whereas three were produced (H_A, H_{HMW}, H_{LMW}). This is in agreement with previous findings by Moona et.al. 2021 (Figure 17).

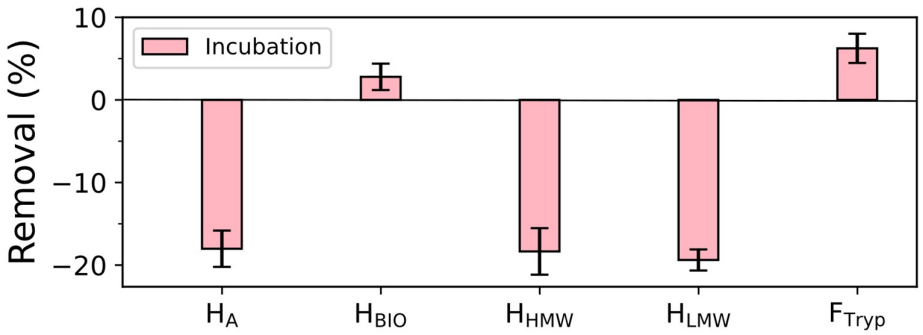

Figure 17: Removal percentages for the five FDOM components (H_A, H_{BIO}, H_{HMW}, H_{LMW}, F_{Tryp}) for before and after incubation (7 ssdays at 35 °C) of water from Göta Älv inoculated with a natural inoculant. Error bars indicate standard errors of means.

The test was extended as a part of the *biofilter performance* sampling campaign. Here, samples were taken before and after biofiltration, assuming this process involved biodegradation. In this full-scale test all components showed some removal, however, more significant removal for H_{BIO} and F_{Tryp} (Figure 18). These samples were collected between February and March 2023 where the temperature of the water was around 2 to 3 °C. The colder temperatures during winter months would cause biological processes to slow down and perhaps the presence of labile organic matter in incoming raw water to be lower.

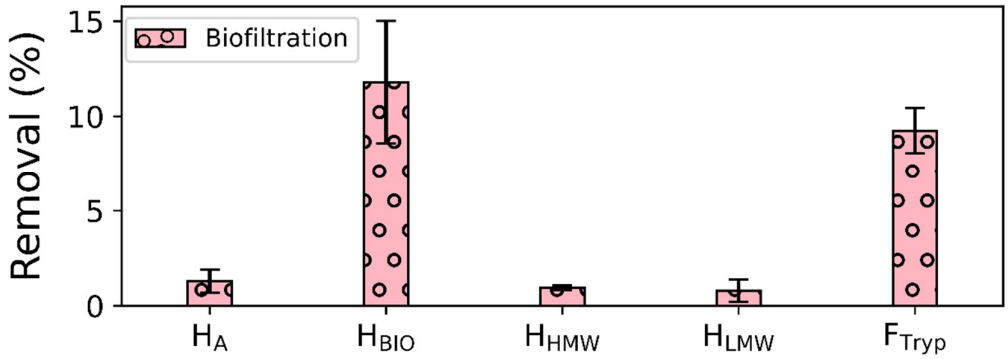

Figure 18: Removal percentages the five FDOM components (H_A, H_{BIO}, H_{HMW}, H_{LMW}, F_{Tryp}) before and after biofiltration at DWTP-K (February-March 2023). Error bars indicate standard errors of means.

Both tests showed that fluorescence measurements could in fact track the removal of NOM through biological processes, moreover these measurements could additionally track production of FDOM fractions. This was an important feature to identify for the future application of mapping out the fate of DOM through water treatment.

Two fractions H_{BIO} and F_{Tryp} in this study, as well as in previous studies were found to track the biological labile organic matter (Moona et al., 2021). These two fractions appear to show a positive correlation (Figure 19) and with the obtained FDOM component no absolute concentrations are obtained, only relative abundances. To avoid duplication only the H_{BIO} fraction will be reported in the *Results and Discussion section*.

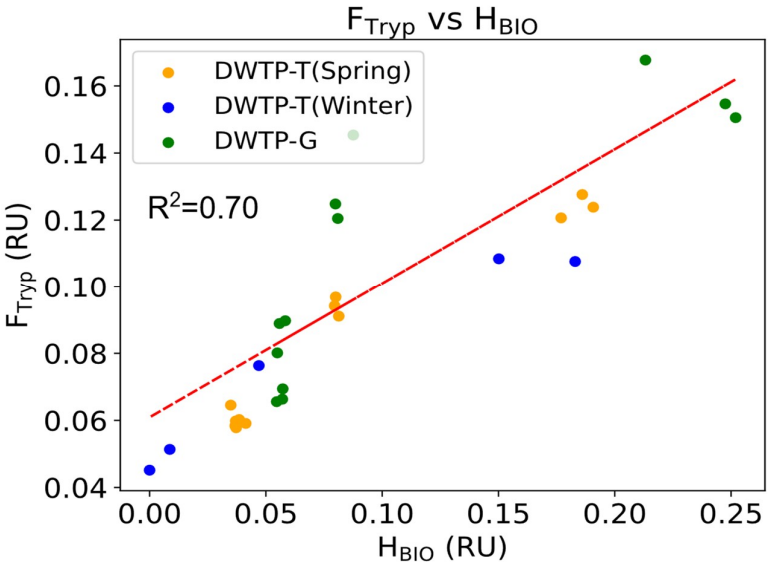

Figure 19: Correlation between the two biodegradable FDOM components F_{Tryp} and H_{BIO}.

Based on these findings the following was concluded and/or confirmed:

- *Biodegradation processes resulted in significant production of the H_A, H_{HMW} and H_{LMW} components and no significant production or reduction of components H_{BIO} and F_{Tryp} .*
- *Differences between the results of the incubation and biofilter test may be due to natural water being collected in September vs February/March, as well as biodegradation taking place at 35 °C and 2-3 °C respectively.*
- *The FDOM components H_{BIO} and F_{Tryp} were affected by biodegradation processes and showed a positive correlation to each other. Based on this correlation only one of the components, H_{BIO}, will be discussed as a tracker for the biodegradable FDOM fraction.*

4.2 Refinement of oxygen sensor method

As previously mentioned, the concept of correlating oxygen consumption to the metabolic activity of bacteria and thus the presence of biodegradable organic matter is not a novel idea. However, here it was attempted to distinguish how the presence of easily metabolised organic carbon affects the initial maximum oxygen consumption rate. As a part of optimisation of the LOCr method, several aspects were considered.

One question that arose was whether the cumulative oxygen consumption of the drop or the oxygen consumption rate of the drop was of interest. Perhaps a combination of the two, cumulative oxygen consumption and oxygen consumption rate would best represent the nutrient utilisation, although it is not yet clear if and how this combination should be interpreted. It was found that oxygen consumption rates more often yielded clear expected trend, based on NaAc calibration curves, compared to the cumulative oxygen consumption. It could be argued that monitoring the consumption rate reduces the effect of slight variation in the cells/mL count added for each replicate. Variations in concentrations of bacteria in samples would perhaps influence the total oxygen consumption more compared to the rate at which the oxygen was consumed. It was therefore decided that the method for now would focus on the labile oxygen consumption rate (LOCr).

Another step in optimising the method was to collect blanks of pure MilliQ. Assuming all samples are exposed to the same level of background noise, one option is to use background subtraction. However, there were technical challenges in implementing blank subtraction due to dissimilar sensor profiles, so it was decided not to focus on absolute measurement and focus on relative results instead.

In summary the following conclusions were made:

- *Oxygen consumption rates resulted in less variability between replicates than for cumulative oxygen consumption.*
- *It was not possible to measure oxygen consumption rates in MilliQ and perform blank subtraction, however it can be assumed that the background noise is the same for all calibrations which allows for comparisons.*

4.2.1 Pretreatment

One aim of the pretreatment for the LOCr was to increase signal recovery. With insufficient signal, the algorithm would not be able to detect the initial exponential oxygen consumption rate, correlated to the bacterial growth. Pasteurisation was investigated to see if it would improve the signal detection of the rapid oxygen consumption. The effect of pasteurisation was included in methods measuring AOC (Li et al., 2017; Van Der Kooij et al., 1982). For the oxygen sensor method pasteurisation resulted in a more focused and easier to distinguish drop in oxygen concentration, Figure 20B. Background noise from pre-existing bacteria may have been removed during pasteurisation, this resulted in more comparable signals between samples, as the bacterial community present during the measurements all stem from the same inoculum.

However, there was a risk that pasteurising the samples not only deactivated indigenous bacteria but could lyse the bacteria. This would result in an additional source of labile organic carbon, which would give false positive signals. Pasteurised and unpasteurised samples were measured with PoP, and no significant change was observed, particularly for the H_{BIO} component (Figure 20A). Through this it was decided to incorporate pasteurisation into the pretreatment.

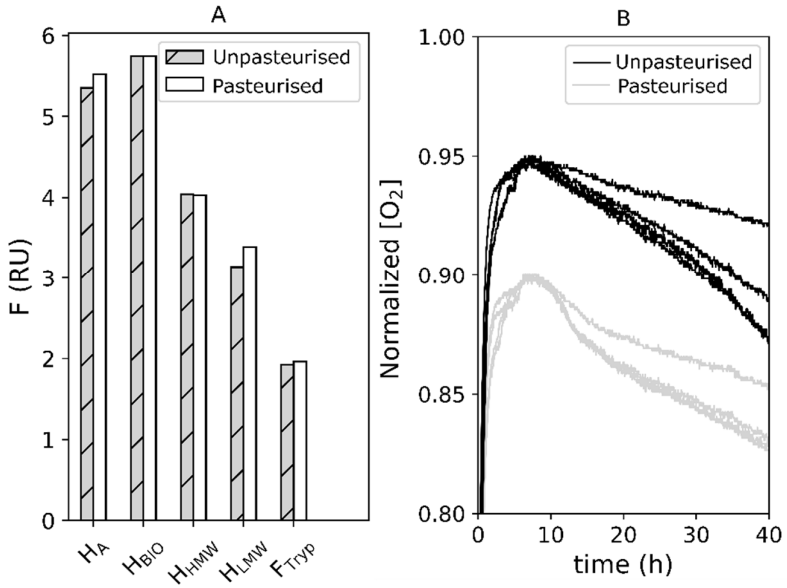

Figure 20: Examples of differences in pasteurised and unpasteurised samples for the five different FDOM components (A) and for oxygen sensor measurements (B).

In summary the following conclusions were made:

- *Pasteurisation improved the signal recovery for the LOCr method.*
- *For the H_{BIO} component in the PoP method, no increase in signal as a result of pasteurisation was observed.*

4.2.2 Inoculum

Bacteria in the inoculant consume the oxygen in the sample and signal the presence of labile organic carbon in the sample. Viable bacteria were therefore essential for producing reliable results. When deciding how to create an inoculant, the aim was to keep the methodology simple and minimise the inoculant to sample ratio.

Active bacteria and a favourable environment were important for obtaining a rapid response in the oxygen sensors. Therefore, it was pivotal to ensure that the inoculum was adapted to the low nutrient concentrations found in drinking water. For incubations, a temperature of 35 °C was selected since it is within the active range for bacteria involved in the decomposition of DOM in natural waters (Donnarumma et al., 2010; Kahli et al., 2022; Schiraldi et al., 2014). Additionally, within the temperature range, higher temperatures are linked to increased metabolic activity (Hall et al., 2008).

The Natural inoculum introduced in the method *section 3.3.1* typically had a low concentration of bacteria, so efforts were made to concentrate the viable bacteria. For this, the collected source water was incubated at 35°C allowing the bacteria to adapt to the temperature at which the experiments were carried out. Initially, the natural inoculant appeared to work but during the colder months, it was difficult to detect any features in the oxygen consumption profile.

Several attempts were made to increase the signal associated with bacterial consumption of labile organic matter without adding more inoculum. One method being centrifuging the inoculant to concentrate the bacteria, which was not successful. Another method involved "boosting" the food in all of the samples in a batch with 1 µg C/L of NaAc, this led to small improvements in the signal recovery. However, a concern with this approach was that adding a labile carbon source might affect the overall carbon consumption pattern. For example, literature has shown that the carbon consumption rate by microbes was lower in solutions where there were mixed carbon substrates compared to solutions containing a single source of carbon substrate. Additionally, the most labile fraction was consumed before less labile fractions were utilised (Harder et al., 1982; Sjöstedt et al., 2022).

Although improvements to signal recovery were obtained using acetate boosting, the seasonal variation in the microbial community continued to present a problem. It was therefore explored if a standardised inoculant could be used in place of the natural inoculum. PolySeed® hydrated in PBS-buffer was tested and found to result in improved signal recovery. It was concluded that the PolySeed® inoculant was superior to using natural inoculant with or without boosting. This was mainly due to the standardisation element it brought to the method, as well as the higher signals, perhaps due to some boosting effect from the seed and to a more active and varied bacteria community.

In summary the following conclusions were made:

- *For the LOCr method, creating a fresh inoculum from natural water prior to each experiment resulted in seasonal variations in the activity of the inoculum.*
- *Boosting samples with a small aliquot of NaAc improved signal recovery, but comparisons between seasons were difficult because the baseline activity of the inoculum varied.*
- *PolySeed® was found to be a promising, standardised alternative to a natural inoculum.*
- *PolySeed® provided an improved signal recovery due to boosting, combined with higher bacteria concentrations and greater standardisation in the method.*

4.3 Drinking water treatment plants

Biofilter performance:
Samples were taken before and after biofiltration at DWTP-K between June 2022 and March 2023. For LOCr no seasonal trends could be investigated as different types of inoculants were used during this period. However, when compiling result for H_{BIO}, changes throughout seasons could be obtained (Figure 21). The highest relative abundance of the H_{BIO} component for incoming raw water was seen during summer months. Moving from summer through autumn and winter a slight decrease in signal was seen, whereas the trend in signal intensities fluctuated a lot when moving from winter to early spring. When looking at the relative abundance of H_{BIO} for treated water after biofiltration the lowest relative abundance of H_{BIO} was seen in November and highest in June/September. Intensities for after biofiltration follow respective trends as for before biofiltration for H_{BIO} and DOC. However, between H_{BIO} and DOC the trends differed slightly.
It was expected that the abundance of labile organic carbon is lowest in the winter months (Health Canada, 2019). Additionally, previous studies of bioavailable fractions in Swedish rivers, suggested that bioavailable fractions are most abundant in April, June, and October, for samples collected January, April, June, August and October (Jones et al., 2023).

When comparing removal percentages for DOC and H_{BIO} during this period, it was seen that the removal percentages for H_{BIO} were constantly higher compared to removal percentages for DOC. It has in previous work been reported that removal detected though fluorescence measurements are usually greater compared to DOC (Philibert et al., 2022). In order to investigate if the greater removal percentages were due to tracking FDOM, the removal percentages for other FDOM components H_{HMW} and H_{LMW} were also plotted. It can be seen that removal percentages for these components are lower than for both H_{BIO} and DOC. Although the labile H_{BIO} fraction is included in DOC, these results suggested that H_{BIO} has the ability to selectively track the removal of organic matter by biofilters, presumably representing the labile organic matter, more selectively.

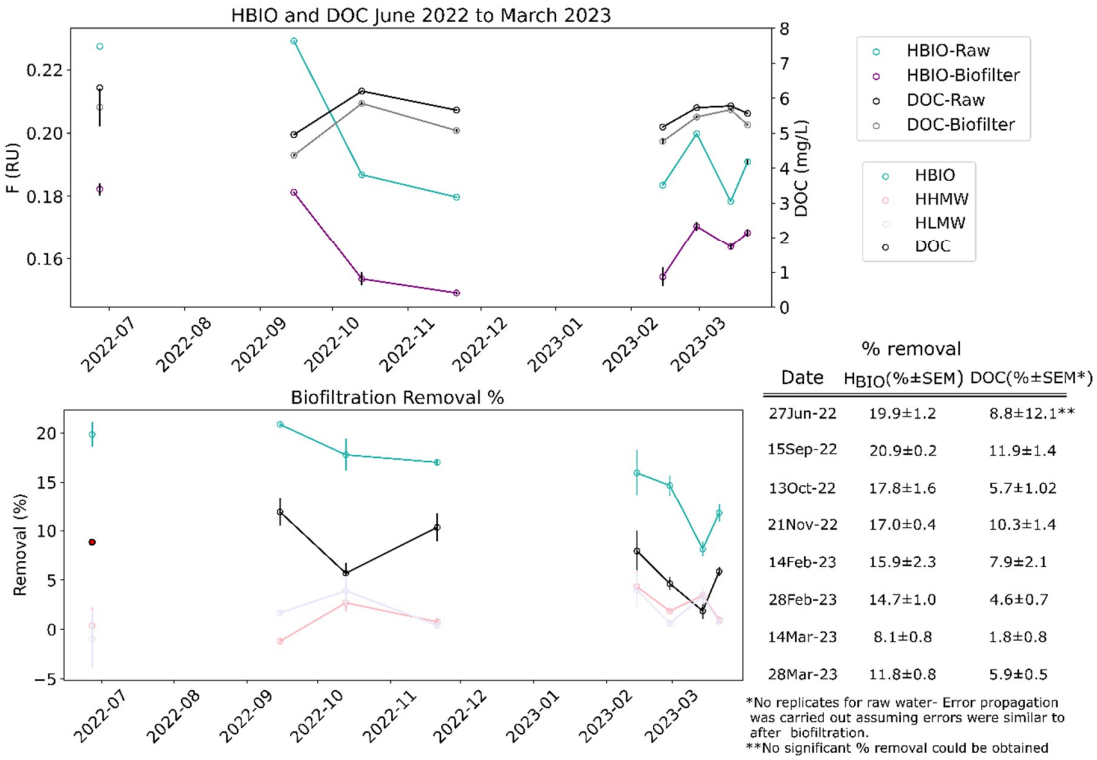

Figure 21: (Top) H_{BIO} and DOC results for sampling campaigns at DWTP-K during the period between June 2022 and March 2023, samples were not taken July-August 2022 and December 2022-January 2023. In green/black H_{BIO}/DOC signals for incoming raw water (Göta Älv) are depicted and in purple/grey after treatment with a biofilter (sand filter). Error bars show standard errors of means. (Bottom) Removal percentages for HBIO (green), DOC (black), HHMW (pink), HLMW (lavender). Error bars show propagated standard errors of means.

DWTP campaign:

Results from PoP and LOCr for four sampling campaigns, involving three DWTPs are show in Figure 22. In DWTP-T the treatment chain was as follows: raw water, flocculation and sedimentation, rapid sand filters, slow sand filters, UV, and chlorination. The treatment process in DWTP-N was as follows: raw water, flocculation, flotation, sand filtration, carbon filtration, UV, and chlorination. Finally, the treatment process in DWTP-G was as follows: raw water, flocculation and sedimentation, carbon filtration, ultrafiltration, and chlorination.

Overall, a decrease of H_{BIO} and LOCr signal intensities was obtained at the end of the treatment compared to the incoming water. This was expected as concentrations of DOC fractions were expected to decrease following successive stages of treatment. However, which fractions are removed efficiently can differ. Sand filters present in DWTP-N (N2) and DWTP-T (T3) are expected to target the labile organic matter, the decreases observed for LOCr and H_{BIO} were therefore expected. After N3 and T3 little to no further decrease was observed for this NOM fraction. Moreover, H_{BIO} component of PoP for DWTP-T showed a similar trend for winter and spring throughout the treatment plant. During spring a slightly higher signal-baseline was seen for the sampling occasion in spring, perhaps due to an increased amount of labile organic carbon.

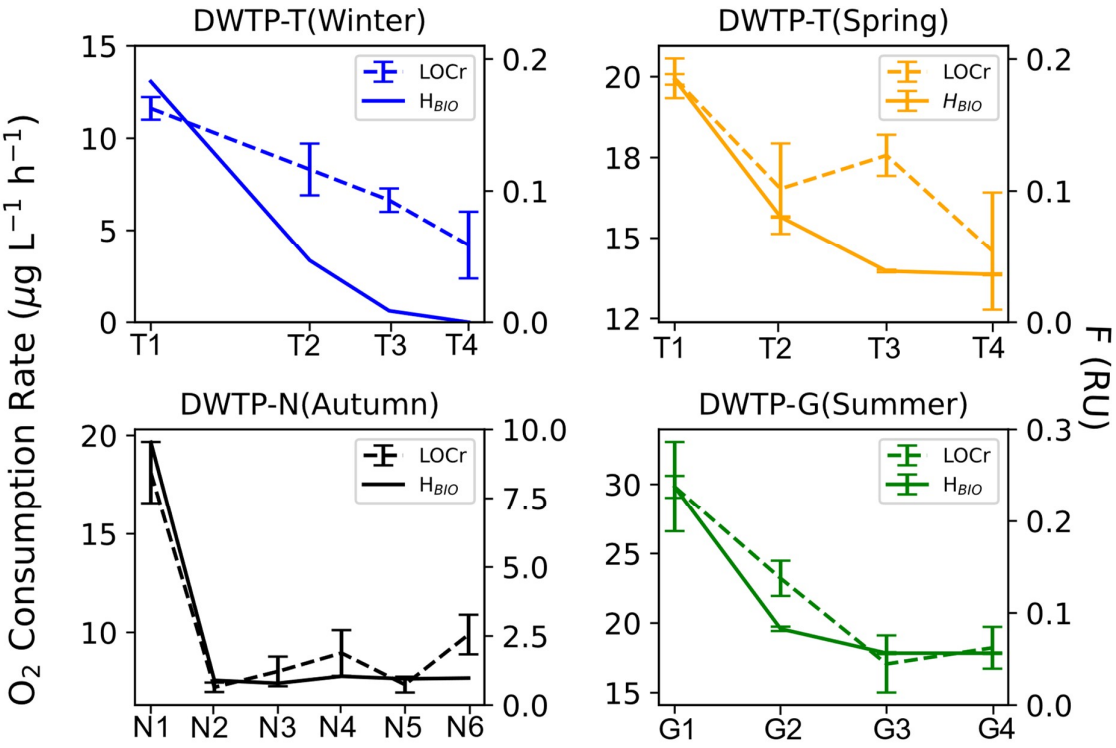

Figure 22: Labile oxygen consumption rate (LOCr) and PoP component H_{BIO} measured after sequential treatment steps in drinking water treatment plants. DWTP-T (January and May 2023): raw water (T1), after flocculation and sedimentation (T2), after rapid and slow sand filters, UV disinfection and chlorination in outgoing water (T3) and in distribution network (T4). DWTP-N in September 2022: Raw water (N1), after flocculation, flotation, and sand filter (N2), after carbon filter (N3), after UV (N4), in outgoing water after chlorination (N5) and in distribution network (N6). DWTP-G in August 2023: raw water (G1), after flocculation and sedimentation (G2), after carbon filter (G3) and after ultrafiltration and chlorination in outgoing water (G4). DWTP-T(Winter) and DWTP-N(Autumn) were inoculated with a natural bacteria community and DWTP-T(Spring) and DWTP-G(Summer) using PolySeed®. Error bars show standard errors of means with propagated standard errors shown for ratios.

As mentioned in the Background *section 2.6.1* PoP could yield information on the relative abundances of biodegradable fractions as well as recalcitrant fractions. Therefore, additional information could be obtained from the sampling campaigns at DWTPs presented above. Firstly, a ratio of the biological labile FDOM (H_{BIO}) and low molecular weight FDOM fractions (H_{LMW}) is presented. It is expected that H_{BIO} components are of the similar size to H_{LMW}, meaning that through physical removal they are expected to behave similarly. However, depicting the ratio of these components could yield information on which treatment steps result in selective removal of the components (Figure 23).

A decrease of the H_{BIO}/H_{LMW} ratio reflects an increased removal of H_{BIO} compared to H_{LMW}, and/or production of H_{LMW}. In contrast an increase in the ratio suggests a more selective removal of H_{LMW}, and/or production of H_{BIO}. Finally, if the ratio remains constant it suggests that both

fractions are removed at the same rate. It was expected that treatment steps involving sand filters would target the labile FDOM fraction, H_{BIO}. The decreased value for the ratio seen in T3 and N2 supported this theory. After T3 (Spring 2023) and N4 little to no change of the ratios were seen, suggesting that H_{BIO} and H_{LMW} were removed at a similar rate.

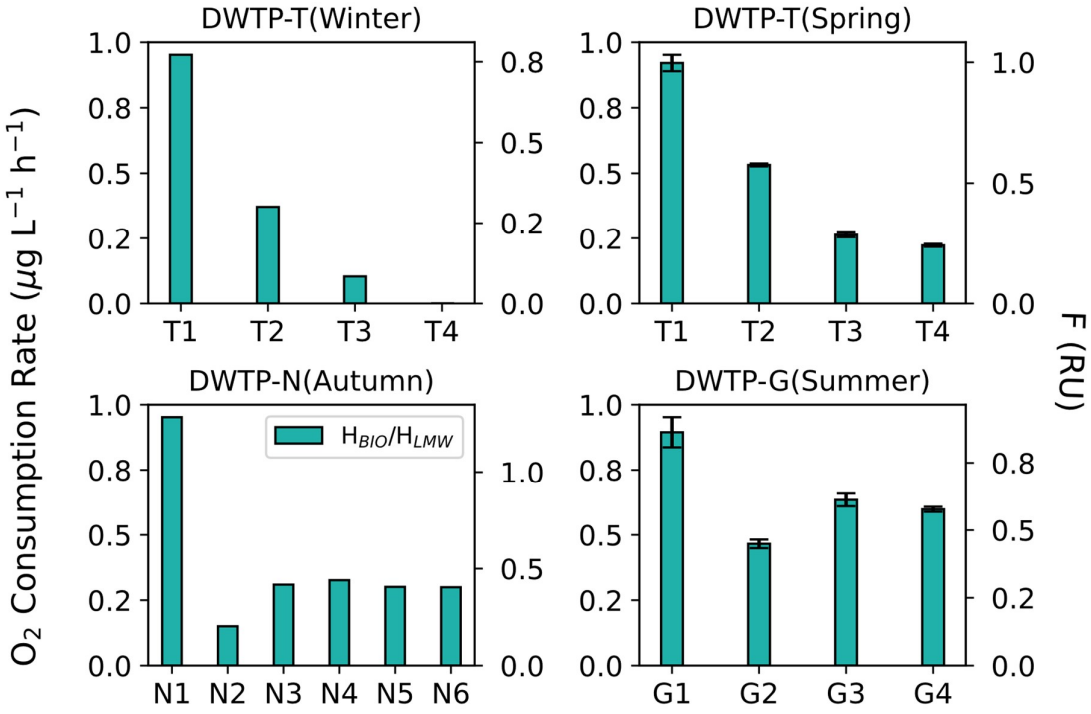

Figure 23: Depicted are ratios of H_{BIO} over H_{LMW} for four different sampling occasions at three different DWTPs. DWTP-T (January and May 2023): raw water (T1), after flocculation and sedimentation (T2), after rapid and slow sand filters, UV disinfection and chlorination in outgoing water (T3) and in distribution network (T4). DWTP-N in September 2022: Raw water (N1), after flocculation, flotation and sand filter (N2), after carbon filter (N3), after UV (N4), in outgoing water after chlorination (N5) and in distribution network (N6). DWTP-G in August 2023: raw water (G1), after flocculation and sedimentation (G2), after carbon filter (G3) and after ultrafiltration and chlorination in outgoing water (G4). DWTP-T(Winter) and DWTP-N(Autumn) were inoculated with a natural bacteria community and DWTP-T(Spring) and DWTP-G(Summer) using PolySeed®. Error bars show standard errors of means with propagated standard errors shown for ratios.

Finally, ratios of H_{BIO} over the high molecular weight component H_{HMW} were also plotted. Similarly, to the previous case: A decrease in the ratio reflects an increased removal of H_{BIO} compared to H_{HMW}, and/or production of H_{HMW}. An increase in the ratio suggests a more selective removal of H_{HMW}, and/or production of H_{BIO}. If the ratios remain constant it suggests that both fractions are removed at the same rate. Again, the steps involving sand filtrations (T3 and N2) showed selective removal of H_{BIO} component. However, the increased value in the ratio for N3 and G3, indicated a greater removal of the H_{HMW} component. The treatment steps T3 and N2 involved treatment with carbon filters, (N3 and G3), which particularly if they are not saturated, are expected to target high molecular weight compounds. On the contrary, no such increase was seen in H_{BIO}/H_{HMW} for DWTP-T, this may be due to the fact that DWTP-T did not include carbon filtration as part of its treatment process.

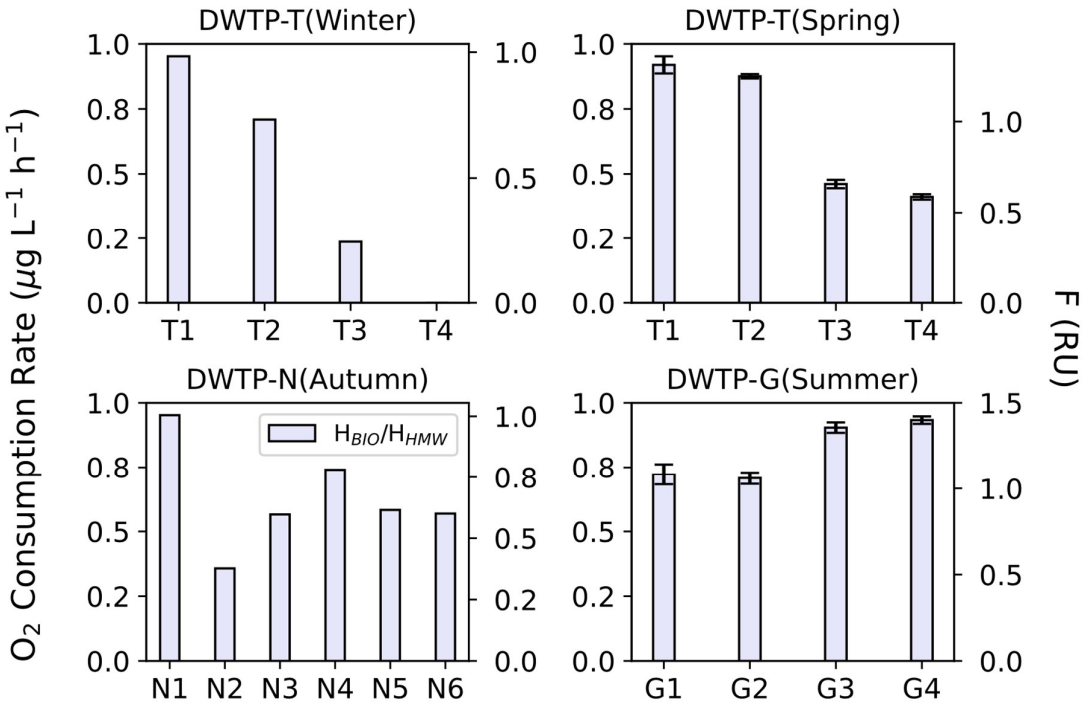

Figure 24: Depicted are the ratios of H_{HMW} over H_{BIO} for four different sampling occasions at three different DWTP. DWTP-T (January and May 2023): raw water (T1), after flocculation and sedimentation (T2), after rapid and slow sand filters, UV disinfection and chlorination in outgoing water (T3) and in distribution network (T4). DWTP-N in September 2022: Raw water (N1), after flocculation, flotation and sand filter (N2), after carbon filter (N3), after UV (N4), in outgoing water after chlorination (N5) and in distribution network (N6). DWTP-G in August 2023: raw water (G1), after flocculation and sedimentation (G2), after carbon filter (G3) and after ultrafiltration and chlorination in outgoing water (G4). DWTP-T(Winter) and DWTP-N(Autumn) were inoculated with a natural bacteria community and DWTP-T(Spring) and DWTP-G(Summer) using PolySeed®. Error bars show standard errors of means with propagated standard errors shown for ratios.

In summary the following conclusions were made:

- *Seasonal variations in the H_{BIO} component of PoP were seen for the period between June 2022 and March 2023.*
- *H_{BIO} component of PoP showed removal throughout the DWTPs.*
- *LOCr showed a similar trend of removal throughout DWTPs, during spring and autumn a signal increase was observed for treatment processes including UV.*
- *Information on selective removal of PoP components could be obtained by assessing ratios.*
- *Selective removal was seen for the H_{BIO} component at sand filter treatment steps.*
- *Selective removal was seen for the H_{HMW} component at treatment steps including carbon filters.*

4.3.1 Comparison with AOC

For the sampling campaigns conducted here, the majority of the commercial P17 results were below detection limit of 5 µg C L^{-1} acetate equivalents, meaning that no significant correlation in trends could be obtained for these amount of data points (Figure 25). However, a weak correlation particularly for LOCr vs P17 could be suggested.

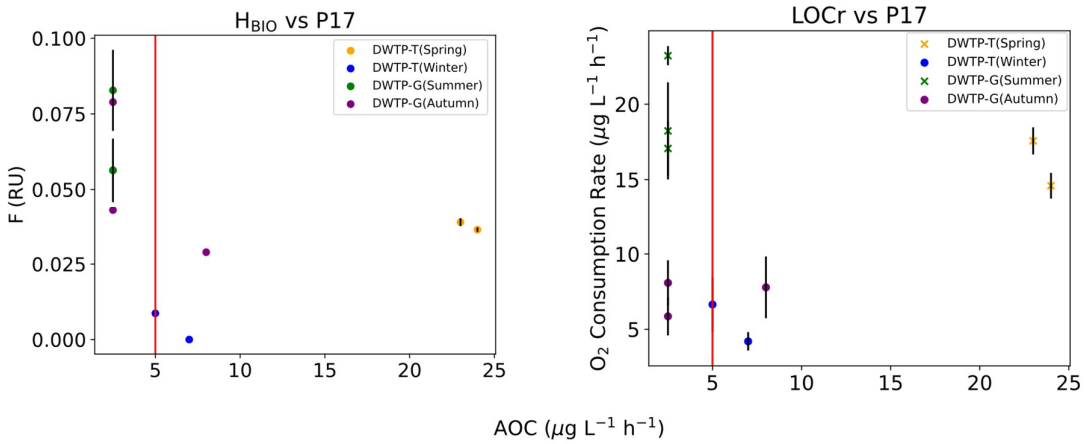

Figure 25: Results for PoP component H_{BIO} and LOCr compared to commercial AOC measurements, for P17. For LOCr crosses(x) signify inoculation with PolySeed® and dots inoculation with a natural inoculant. Error bars show standard errors of means.

In summary the following conclusions were made:

- *No significant correlation between the commercial method and LOCr/PoP could be obtained from this sampling campaign. However, an indication of a weak positive correlation could be seen.*

5. Conclusions

To conclude, tests were conducted to evaluate if the theory behind LOCr and PoP applied in practice. For LOCr dilutions of NaAc and natural water were measured. These results showed an increase in oxygen consumption rate with an increased concentration of labile organic matter. For PoP the initial tests concluded that the signal strengths for the biodegradable components, H_{BIO} and $F_{Tryp,}$ decreased as a result of biodegradation processes.

Work was carried out to refine the LOCr method and increase signal recovery for the method. It was found that pasteurising the sample before inoculation helped the algorithm to identify the duration at which the maximum initial oxygen consumption takes place. As a result, pasteurisation was implemented in the method. Additionally, standardising the inoculum permits the method to be less effected by seasonal variations of bacterial activity of the natural inoculum.

For the third phase in the evaluation of LOCr and PoP the applicability of these methods in full-scale DWTPs were evaluated. First, seasonal trends of the labile PoP component H_{BIO} in raw water and after treatment with biological filter were evaluated and compared to DOC results. Slight variations in trends suggest that the H_{BIO} measurements give other information than that contained in DOC measurements.

LOCr and PoP were used to track the labile fraction throughout the treatment processes, both methods showed a general decrease of this fraction throughout the DWTPs. Further information from PoP could be extracted, though evaluating ratios of FDOM components. For this project, selective removal of the biodegradable fractions, H_{BIO} was evaluated with regards to high and low molecular weight fractions, H_{HMW} and H_{LMW} respectively. Results from these evaluations showed selective removal of H_{BIO} for stages including biological filters, and selective removal of H_{HMW} for treatment steps involving carbon filters.

In the following points the conclusions are presented as answers to the research questions proposed in *section 1.1.*

1) *Can methods based on oxygen sensors and fluorescence measurements be employed for quantifying labile organic carbon in water systems?*

Overall, LOCr and PoP measurements indicated an increase in signal with respect to an increase in the abundance of labile organic carbon. The two methods could also identify a reduction in signal strength due to biodegradation processes. Further suggesting that these two methods could track the labile fraction of NOM.

2) *What role could these methods have in the future monitoring of labile organic carbon to assess the efficiencies of water treatment steps?*

LOCr and PoP were found to track labile organic carbon. Based on this property both methods could aid in understanding the fate of this fraction throughout the drinking water treatment process. The methods could also be employed for comparing removal efficiencies of this labile fraction. When comparing parallel filter beds, the methods could facilitate detection of reduced removal efficiencies.

Seasonal variations could be obtained with PoP. This could be of relevance for DWTPs for detecting deviating trends, and when appropriate action to mitigate an increased level of labile

organic carbon must be taken. Obtaining this information may also be possible with LOCr when using a standardised inoculum.

LOCr and PoP detect the labile fraction of NOM based on two different approaches, detecting biological activity and characterising NOM based on chemical structure respectively. This gives the methods different qualities. LOCr relies on viable bacteria in order to work, whereas it at the same time could yield in information on the utilisation of the substrates present in the water sample. Whereas PoP does not consider the utilisation of substrates in the water but rather it is based on structural properties and can though this give a first order estimate of the lability of DOM.

It is important to note that the PoP method gives a relative abundance of FDOM components and not an absolute concentration. Furthermore, all labile organic matter is not encompassed in the H_{BIO} component, however results suggest that this FDOM component can act as a proxy when discussing the fate of this fraction.

PoP measurements had the additional benefit that more information could be obtained from a single measurement. By utilising the other FDOM components obtained with PoP, information in removal and production of these components could be obtained. These pieces of information could be very beneficial for DWTPs in understanding what happens to different fractions of NOM at a particular treatment step.

6. Future research

This thesis lays the groundwork for understanding the applicability of LOCr and PoP methods for quantifying labile organic carbon. Further research should further assess the applicability of these methods.

When using the LOCr method with PolySeed® as a standardised inoculum, it may be possible to study seasonal variations in the abundance of labile organic carbon in water resources more cheaply than before. Prior to this, it would be informative to monitor what occurs at the microbial level during LOCr measurements by combining flow cytometry measurements with the LOCr method. Flow cytometry can give information of the abundance of and condition of cells of the bacteria consuming the oxygen during the LOCr methods. This information could be beneficial at sample preparation stages and throughout the LOCr measurements. For example, flow cytometry could be used to evaluate if bacteria are still intact after the pasteurisation process, confirming what was found with fluorescent measurements. Additionally, flow cytometry results from samples taken at different stages of the LOCr measurements could yield information on the correlation between oxygen consumption rate and bacterial regrowth. Another aspect to explore further is the effect of carbon substrate composition on measured metabolic activity using the LOCr method, which is important if the aim is to obtain an absolute carbon concentration in acetate equivalents.

Using the PoP method, the ability to detect selective removal and production of labile DOM yields valuable information on the overall treatment process by identifying which treatment processes efficiently remove certain FDOM fractions, and which treatment steps potentially produce other types of NOM. This knowledge would help DWTPs compare the efficiency of treatment steps and detect changes in treatment performance. That information would assist in identifying when to take appropriate action to optimise the treatment steps. It would be beneficial to compare if the same trends are shown for result obtained by a pre-existing PARAFAC model compared to results from a customised model developed for the water studied. However, the practical applications and limitations of the PoP method requires further exploration.

Evaluating LOCr and PoP in further applications is important for understanding the reliability of the methods. The results from this project indicate there is potential for using these methods to track labile organic carbon in water. However, additional biodegradation processes and waters containing different NOM compositions should be studied. A larger study measuring trends and seasonal variations using the LOCr and PoP methods would give a greater picture of what information can be obtained. Comparing these results to other methods for tracking NOM, such as DOC, UV-Vis spectroscopy and specific UV absorbance (SUVA), and looking at how they differ from each other could improve the understanding of the fate of labile DOM in raw and treated water.

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