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Peracetic Acid Disinfection in Freshwater Recirculatory Aquaculture Systems

Comparison of consumption of peracetic acid in different sample systems

Anushree Mainali
Master of Science in Aquaculture

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Main supervisor: Professor Odd-Ivar Lekang, REALTEK

Co-supervisor: Professor Dr. Vasco C. Mota, REALTEK

Faculty of Science and Technology

Department of Mechanical Engineering and Technology Management

Norwegian University of Life Sciences (NMBU)

Norwegian University
of Life Sciences

Preface

I am pleased to present this master's thesis, which was completed at the Norwegian University of Life Sciences (NMBU) in the Department of Mechanical Engineering and Technology Management. This thesis is a requirement for the completion of my two-year Master of Science in Aquaculture program at NMBU. The experimental and writing work for this thesis was carried out during the Spring semester of 2023.

I would like to express my gratitude to my thesis supervisors for providing valuable guidance, advice, and support throughout the project. Additionally, I would like to thank all those who contributed to this work and supported me throughout my academic journey.

Ås, May 14th, 2023.

Anushree Mainali

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Last but not the least, I would like to thank the universe for conspiring in my favour and bringing all the right people and opportunities into my life. This thesis is a testament to the power of positive thinking, hard work, and never losing sight of one's goals.

Thank you all from the bottom of my heart.

A.M.

Abstract

Peracetic acid (PAA), a powerful organic peroxide, is regarded as a rather sustainable disinfectant in aquaculture due to its broad efficacy against various pathogens at low concentrations. PAA-based disinfectants are highly regarded in recirculating aquaculture systems (RAS) due to their low risk of bioaccumulation, rapid degradation with neutral residuals, and minimal impact on biofilter performance. However, the unknown no-observed-effect concentration in Atlantic salmon parr remains a concern.

In this study, it is attempted to investigate the decay kinetics of PAA as a disinfecting agent in aquaculture by comparing the consumption of PAA across the analysed samples at two temperatures. The consumption of PAA in two types of sample systems; a. PAA-adapted and PAA-naive RAS water samples, and b. samples with seven levels of feed loads were studied and compared. The effect of PAA treatment on the water quality parameters of TSS, COD and turbidity at two temperatures 12°C and 15°C were quantified and compared as well.

Regardless of the treatment temperature, PAA decayed more rapidly in PAA-adapted RAS water samples compared to PAA-naive samples. Higher PAA decay was observed in samples with higher feed loads. Furthermore, PAA treatment resulted in an increase in COD and a significant decrease in TSS and turbidity. The results indicated that the treatment temperature shows a positive temperature-decay correlation for PAA, this was majorly due to the comparatively rapid consumption of PAA immediately after the treatment at 15°C than at 12°C. The effect of temperature on water quality parameters was considerable, with higher temperatures resulting in more significant changes. In summary, the findings of the study can aid in supporting the pulse application of PAA in freshwater RAS because of its implications of an adapted sample system. It also elucidates the contribution of treatment temperature and feed loads in PAA decay.

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Abbreviations

BOD : Biochemical Oxygen Demand

CO_2 : Carbon dioxide

COD : Chemical Oxygen Demand

DI : Deionized

DO : Dissolved Oxygen

DPD : N, N-diethyl-p-phenylenediamine sulphate

FNU : Formazin Nephelometric Units

H_2O_2 : Hydrogen peroxide

NS-EN : Norsk Standard - Europäische Norm

NTU : Nephelometric Turbidity Units

ORP : Oxidative reduction potential

PAA : Peracetic acid (CH_3CO_3H)

RAS : Recirculating Aquaculture System

SS : Suspended Solids

TDS : Total dissolved solids

TSS : Total Suspended Solids

1. Introduction

1.1 Background

Aquaculture is the fastest-growing food-producing industry, with increased production of farmed seafood over the last decade highlighting the role of aquaculture in meeting the rising consumer demand. Aquaculture production is expected to reach 109 million tonnes in 2030, representing a 32% increase after 2018 (Stankus, 2021). Modern aquaculture development is geared towards sustainably increasing productivity. Controlling the rearing environment to give fish the best environment for growth and a thriving habitat that supports excellent welfare is essential for successful and sustainable aquaculture (Cabillon and Lazado, 2019).

Recirculating aquaculture systems (RAS) consists of a well-coordinated set of complementary processes that enable a portion of water to leave a fish culture tank to be treated and then reused in either the same tank or other fish culture tanks. They are designed to minimise the environmental impact of fish farming by reusing water (Timmons et al. 2002). They can function with varying degrees of recirculation by reducing the volume of makeup water required (Lekang, 2007). They are particularly considered in commercial scale food-fish production due to their perceived benefits such as greatly reduced land, reduced production costs by optimising energy required to maintain a specific temperature and water conditions, and the feasibility of locating in close proximity to top markets (Dunning et al., 1998).

However, there are a few downsides of RAS, most important is the deterioration of water quality due to improperly controlled water treatment systems. Due to this the risk of disease outbreaks is inherent in the use of RAS (Liu et al., 2017). The water quality in RAS depends on several factors such as the source, the level of recirculation, the species have been cultured and the water treatment process within the system (Losordo et al., 2000). According to Sanni and Forsberg (1996), the majority of water quality issues in RAS are caused by low dissolved oxygen (DO) concentration in the culture water and high concentrations of fish wastes.

Depending upon the aquatic species, water quality requirement, and water use intensity, RAS in general consists of multiple of the following treatment processes (Losordo et al., 2000).

- Mechanical filtration units to remove particulate solids.
- Biological filtration units to remove ammonia.
- Aerators/strippers to add dissolved oxygen and decrease carbon dioxide or nitrogen gas levels closer to atmospheric saturation.
- Oxygenation units to increase DO concentrations above atmospheric saturation levels.
- Disinfection units such as ozonation units or UV irradiation.
- pH controllers to add alkaline chemicals for maintaining water buffering.
- Heaters or coolers to maintain desired water temperature.

Utilising components of cost-effective water treatment systems is a crucial element of a successful RAS (Dunning et al., 1998). Several factors such as feeding strategy, diverse environmental conditions and various disinfection methods influence the water quality in RAS as shown in Figure 1 (Li et al., 2023).

Disinfection is an integral component in RAS. The potential proliferation of pathogenic microorganisms in the culture water is detrimental to the growth and development of reared fish or cultivated organisms. So, it is necessary to disinfect pathogenic microorganisms from the water in advance to stop the spread of diseases (Blancheton et al., 2013). There are several methods of disinfection such as ozonation, UV irradiation, hydrogen peroxide (H_2O_2), peracetic acid (PAA), chlorine to name a few.

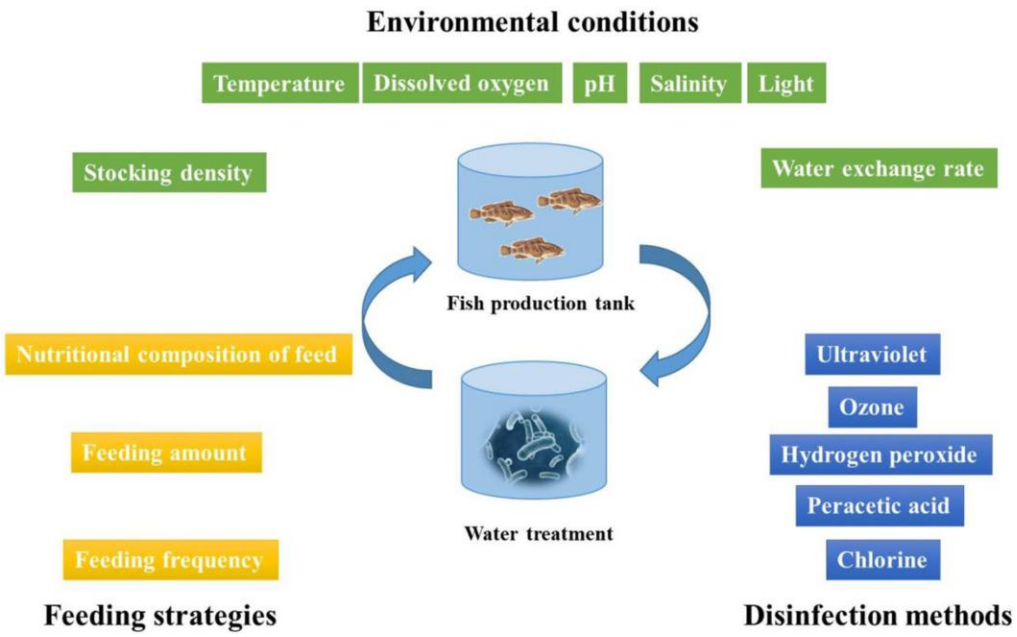

Figure 1. Influence factors in a Recirculatory aquaculture system (Source: Li et al., 2023)

The most widely used disinfection techniques in RAS are ozonation and UV irradiation (Timmons et al., 2018). However, in recent years other disinfection techniques have been introduced in RAS such as addition of PAA and H_2O_2.

At low doses, the effectiveness of peracetic acid (PAA) against several fish diseases has been demonstrated by Pedersen et al., (2013). A study by Pedersen et al., (2009) suggested that when using PAA in RAS, the biofilter should be bypassed since PAA concentrations as low as 2 mg/l can affect the nitrifying bacteria. This study outlines a quick alternate method for adding PAA to a RAS to ensure the biofilter's safety (Pedersen et al., 2009). Because PAA spontaneously breaks down and leaves no toxic residues behind, its use is regarded as environmentally friendly (Wagner et al., 2002). While using PAA, fish welfare should also be considered. Prior research on fish welfare has primarily concentrated on the acute toxicity of PAA or the mortality or gill damage brought on by in vitro treatments (Straus et al., 2012). However, the concept of fish wellbeing goes much beyond just preventing mortality (Pedersen et al., 2013).

Knowledge of the decay kinetics and fate of active residues is necessary for the development of new water disinfection procedures and chemotherapeutic drugs using water as a delivery matrix in order to ensure safe and efficient treatment regimens and to assess the potential negative environmental impact on the receiving water bodies (Pedersen and Lazado, 2020). Without an appropriate method to confirm the actual residual concentration of interest, controlling the quantity and reactivity of PAA poses the biggest obstacle to the efficient use of PAA-based products (Kitis, 2004). It is difficult to provide general guidelines when the delivered and expected quantities have not been measured. Additionally, a significant overdose could harm fish health because PAA is such a potent oxidant. (Pedersen et al., 2013) The observed differences in PAA treatment efficacy can very well be explained by the consumption of PAA (Rintamäki-Kinnunen et al., 2005). Simple analytical techniques are required to determine how residual PAA will behave in aquaculture systems. Application of PAA in adequate doses on commercial fish farms is more difficult than the application of products like formalin or salt due to the fact that PAA half-lives, as observed in on-farm measurements, are on the order of a few minutes. (Pedersen et al., 2010)

1.2 Objectives

The focus of this study was to determine and understand the decay kinetics of peracetic acid as a disinfecting agent in different water samples.

The primary objective of this thesis was to investigate PAA decay when used as a disinfectant in aquaculture.

In order to achieve the main objectives, the following **Sub-objectives** were formulated:

I. To quantify and compare the decay of peracetic acid in freshwater RAS samples; one each from an adapted system and from a naive system, at two treatment temperatures.

II. To quantify and compare the decay of peracetic acid in samples with different loads of feed to understand the effect of feed in the decomposition of PAA in freshwater RAS at two temperatures.

III. To test and quantify the effect of peracetic acid treatment on several water quality parameters like, total suspended solids, chemical Oxygen demand, and turbidity at two temperatures.

The following **Hypotheses** were tested in order to achieves the objectives.

Hypothesis A

H0 There is no difference in the decay rate of PAA in an adapted and a naïve sample.

H1 There is a significant difference in the decay rate of PAA in an adapted and a naïve sample.

Hypothesis B

H0 The presence of feed as an organic matter in the water doesn't affect the decay rate of peracetic acid.

H1 The presence of feed as an organic matter in the water significantly increases the decay rate of peracetic acid.

Hypothesis C

H0 There is no change in the water quality parameters of COD, TSS, and turbidity as a result of PAA treatment at two treatment temperatures.

H1 Water quality parameters like COD, TSS, and turbidity significantly change as a result of PAA treatment at two treatment temperatures.

2. Literature Review

2.1 Water quality in RAS

Water quality standards for aquaculture systems have historically considered variables like temperature, dissolved Oxygen, total gas pressure, ammonia, and nitrite. Numerous published criteria were developed to protect the environment for a variety of species and life stages (Colt, 2006). Fine particles, refractory organics, surface-active chemicals, metals, and nitrate may all play a significant role in water reuse systems. The operation of unit processes, as well as criteria for the culture species, are two main categories of water quality criteria that are required for reuse systems (Colt, 2006).

The important factors in the selection of water quality for aquaculture systems can be divided into (i) lethal effects, for example, it is necessary for the design of backup systems, and transport systems, (ii) sub-lethal effects such as growth, fin quality and appearance, tissue quality, physiological quality, behavioural and health of the fish, and (iii) regulatory requirements, for instance, phosphorous, nitrogenous compounds, suspended solids, chemical releases, biochemical Oxygen demand, tracing of contaminants in tissue (Colt, 2006).

2.1.1 Water quality requirements for salmonids

Salmonids, which include trout and salmon, have specific needs in terms of water quality to survive and thrive. According to Terjesen et al (2013), the particular focuses required to determine the water quality for salmon production in RAS to achieve optimal performance, health and welfare of the fish are (i) safe chronic ammonia and nitrite levels in RAS, (ii) effects of fish density determined in RAS environments, (iii) thermal optima for low malformation rates of salmon in RAS, as have been studied in FT (Bæverfjord et al., 1999), and (iv) optimal tank water velocity during parr production in RAS.

Salmonids are cold-water fish that have specific temperature needs when growing (10-15°C to thrive and reproduce) (Welch et al, 1995). Fish and other organisms in their environment are

affected by water temperature in terms of their metabolism, behaviour, and mortality (Mihursky and Kennedy 1967). Even though fish can survive at temperatures that are close to the extremes of their preferred range, growth is slowed at low temperatures due to the slowing of all metabolic processes and at high temperatures due to the need to use most or all the food for maintenance. Many salmonids alter their behaviour in response to temperature changes. (Welch et al, 1995) Temperatures exceeding 20°C can be uncomfortable, and in some cases fatal, to some species (Carter, 2005b).

Salmonids may be able to survive in environments with DO levels less than 5 mg/l (Carter, 2005a). Various species of salmon and trout need at least 6 mg/l of DO to maintain their metabolic activities and general health. However, for optimum development and survival, DO concentrations above 8 mg/l are preferred. Salmonids can experience stress and possibly death in severe situations when the DO concentration is below 5 mg/l. (Davis, 1975; Lekang, 2007). The survival threshold concentration for Atlantic salmon smolts is approximately 3.3 mg/l (Alabaster et al. 1979), and for rainbow trout, the threshold concentration is as low as 2 mg/l according to laboratory tests (Alabaster et al. 1957). However, concentrations as low as 5 mg/l are likely to limit growth rate and food conversion efficiency.

Salmonids prefer water that is neutral to slightly acidic, with a pH of 6.5-8.0. (Edward and Hjeldnes, 1977). Because of its toxicity to salmonids at high concentrations, water quality must be maintained to keep ammonia levels below 0.02 mg/l and nitrite levels below 0.5 mg/l (Summerfelt, 2000; Terjesen et al., 2013). Turbidity, or cloudiness in the water, can impair visibility, making it more difficult for salmonids to find food and evade predators. Salmonids can be harmed by high levels of TDS, which includes salts and minerals. TDS values of more than 1,000 mg/l are generally regarded as inappropriate for salmonids. (Scannell et al, 2001)

Because salmonids can be sensitive to heavy metals and other pollutants in water, water quality must be monitored and managed to reduce exposure to these contaminants. Overall, maintaining high water quality is critical for salmonid health and survival (Colt, 2006).

The composition and stability of the inlet-water source are essential input parameters in production, and the site-specific inlet-water quality determines water treatment strategies (Kristensen et al., 2009). Norwegian aquaculture has documented several examples of the effects of suboptimal inlet water quality (Åtland et al., 2007), and a variety of water treatment strategies are employed, including catchment (Teien et al., 2005a), silica-lye addition (Teien et al., 2006), seawater addition (Rosseland and Skogheim, 1986) and lake/river (Teien et al., 2005a). Table 1 lists the recommended limits of the water quality parameters for the stressors that are commonly studied for Atlantic salmon farming (Da Silva et al 2018).

Table 1. Recommended water quality requirement of recirculating aquaculture system for Atlantic salmon (Masser et al., 1999; cited after Da Silva et al., 2018)

Parameters	**Recommended value or range**
Temperature	The optimum range for species cultured with less than 4°F as a rapid change
Dissolved oxygen	60% or more of saturation, usually 5 ppm or more for warm water fish
Carbon dioxide	Less than 20 ppm
pH	7.0 to 8.0
Total alkalinity	50 ppm or more
Total hardness	50 ppm or more
Un-ionized ammonia	Less than 0.05 ppm
Nitrite	Less than 0.5 ppm
Salt	0.02 to 0.2 %

2.1.1.1 Total suspended solids in aquaculture

Total suspended solids (TSS) can be defined as the mass (in mg) or concentration (in mg/l) of organic and inorganic matter which is retained in the water by turbulence (Bilotta and Brazier, 2008). They typically consist of fine particles with a diameter of less than 62 µm and are measured directly by the collection of sample water followed by filtration of this sample through a dried and pre-weighed 0.7 µm pore-size glass fibre filter (Gray et al., 2000).

The deterioration in water quality can be caused in several ways by the suspended solids. Physically, TSS is highly likely to result in the reduction of penetration of light and change in temperature (Ryan, 1991). Chemically, due to the presence of TSS, it is possible that

contaminants may be released into the water for example, heavy metals and pesticides (Dawson and Macklin, 1998). Additionally, if TSS consists of high organic content, the DO will be consumed by in situ decomposition followed by a decrease in the concentration of DO and, in terms, be fatal for fish (Ryan, 1991).

Furthermore, TSS can directly affect free living fish by clogging and being abrasive to fish gills (Cordone and Kelley, 1961), or stressing the fish and destroying their immune system. This will escalate fish disease susceptibility and osmotic dysfunction (Redding et al., 1987). The presence of TSS also influences the migration of wild Salmons (Bisson and Bilby, 1982). The influence of TSS on fish is dependent on these four major factors: chemical composition of TSS, concentration of TSS, duration of exposure to TSS, and particle-size distribution of TSS (Bilotta and Brazier, 2008). Moreover, the real effects on salmonids will also vary based on the life stage of salmon (Bash et al., 2001).

2.1.1.2 Turbidity in aquaculture

Turbidity is a measurement of the light-scattering properties of water. Numerous probes are available to measure turbidity. Due to low cost and ease of use, Nephelometric turbidity metres have been most widely applied in field studies, and turbidity data are recorded in Nephelometric Turbidity Units (NTU) (Lewis, 1996). Similar to NTU, the scattered light from the sample is measured using Formazin Nephelometric Units (FNU) at a 90-degree angle from the incident light. When referencing the turbidity method ISO 7027, FNU is most frequently used (Buzoianu, 2000). In aquaculture, turbidity is considered a parameter of immense importance as it can have adverse effects on water quality and fish health (Pedersen et al., 2017). There are correlations and differences between suspended solids and turbidity. Suspended solids are the actual measure of the amount of sediment suspended in the water column, the determination process of which is complex and time-consuming. While turbidity is the measure of the refractory characteristic of materials in water. So, there are many limitations when using turbidity as a surrogate measure of SS (Bilotta and Brazier, 2008). Because besides concentrations of TSS, turbidity is also influenced by the particle-size distribution, the shape of particles and other dissolved materials (Sorensen et al., 1977). Studies have shown that turbidity levels beyond

natural background can affect the physiology and behaviour of salmonids (Gregory and Northcote, 1993). Exposure to high levels of suspended solids may be fatal to salmonids, while lower levels of suspended solids and turbidity will also lead to chronic sub-lethal effects such as loss or reduction of foraging capability, reduced growth, and reduced resistance to disease (Lloyd, 1987). Table 2 lists the effects of turbidity on salmonids' behaviour (Bash et al., 2001).

Table 2. Effects of turbidity on Salmonids behaviour (Bash et al., 2001)

Physiological	**Behavioural**	**Habitat**
Gill Trauma	Avoidance	Reduction in spawning Habitat
Osmoregulation	Territoriality	Effect on Hyporheic upwelling
Blood Chemistry	Foraging and Prediction	Reduction in BI habitat
Reproduction and growth	Homing and migration	Damage to Redds

2.1.1.3 Chemical oxygen demand in aquaculture

Chemical Oxygen Demand, or COD, is an indicator of how much organic matter in water can be oxidised by potent oxidising agents (APHA, 2017). It is a commonly used metric to determine the amount of organic contamination in wastewater. COD is expressed in units of mass per volume, typically milligrams per litre (mg/l) or parts per million (ppm). The standard unit for COD is mg/l (Tchobanoglous et al., 2014). The COD test uses potassium dichromate in an acidic media to oxidise organic materials in a water sample. The amount of organic matter in the sample is calculated using the change in dichromate ion concentration ($Cr_2O_7^{2-}$), which is determined as a result (APHA, 2017). The COD test is often used to assure compliance with discharge limitations and assess the effectiveness of the treatment processes in wastewater treatment facilities (Tchobanoglous et al., 2014).

Variations in COD levels in recirculating aquaculture systems (RAS) can significantly affect Atlantic salmon growth rates and general health (El-Sayed et al., 2019). Fish that are exposed to high levels of COD may experience stress and decreased feeding activity as a result of the water's lower DO content (Chen et al., 2018). Furthermore, high COD concentrations can encourage the development of pathogenic bacteria in RAS systems, raising the likelihood of disease outbreaks (Wedemeyer and Yasutake, 1997). Researchers have suggested using a variety of treatment

techniques, such as activated carbon filters or ozone treatment, to address the problem of COD fluctuation in RAS (Huang et al., 2020).

The detrimental effects of COD changes on fish health and growth can also be lessened by maintaining ideal water quality parameters such as pH, temperature, and DO levels (Timmons et al., 2018).

2.1.1.4 Temperature in aquaculture

The influence of temperature in RAS impacts several physicochemical parameters and is therefore essential to quantify during water quality designation. The solubility and reaction rates of chemicals present in the water are affected by variations in temperature. It is highly likely that elevated temperatures can increase the solubility of toxic compounds like ammonia. It can also lead to an increase in the solubility of salts in the water, followed by an increase in salinity and conductivity. A rise in temperatures can also lead to the lower solubility of dissolved gases, such as DO levels and CO_2 levels. (Gray, 2000) High CO_2 levels combined with low temperatures (5°C) have been shown to inhibit the growth of Atlantic salmon compared to high CO_2 at high temperatures (15°C) (Thorarensen and Farrell, 2011). Typically, the optimum temperature levels in RAS for Atlantic salmon should lie between 12 $^{\circ}$C to 14°C (Dalsgaard et al., 2013).

2.2 Requirement of disinfection in freshwater RAS

Disinfection is a critical component of establishing effective biosecurity by preventing pathogen introduction and transmission within and between aquaculture facilities (Summerfelt et al., 2009). As mentioned earlier, ozone addition and UV irradiation are the most used methods of disinfection in RAS. Some other methods include the use of peracetic acid, hydrogen peroxide, chlorine to name a few. There is a lack of documentation on disinfection practices and strategies, making it challenging to determine optimal approaches and benchmark the current status (Lazado and Good, 2021). Failure to implement internal disinfection processes can result in the accumulation of fish pathogens, especially during disease outbreaks when the pathogen is propagating and shedding from its host (Summerfelt et al. 2009).

Although producers acknowledge the importance of disinfection and various recommended guidelines exist (Yanong and Erlacher-Reid, 2012; Fiskeridepartementet, 2008; OIE, 2003), there is still a lack of documentation on the current status of disinfection strategies in aquaculture, particularly in Atlantic salmon aquaculture where production technologies have advanced significantly in recent years (Terjesen et al., 2013; Ytrestøyl et al., 2020). As many facilities are now using recirculating aquaculture system (RAS) technologies, a reassessment of current disinfection practices is necessary to ensure that protocols are adaptive to the present demands of the systems and production practices. This would allow benchmarking, streamlining, and identification of critical points for revision and recalibration (Lazado and Good, 2021).

Carlo C. Lazado for Nofima and Chris Good for The Conservation Fund's Freshwater Institute recently published a scientific paper describing the differences and similarities in disinfection practices at 25 modern RAS facilities in Norway and North America (US and Canada) based on a survey, to provide information that can be used to develop new standards to improve efficacy and prevent disease outbreaks in a rapidly expanding industry. All respondents emphasised the importance of effectiveness against pathogens and user safety when choosing disinfectants. The survey found that while different disinfectants are used at all facilities, peracetic acid-based disinfectants are primarily used in Norway, along with chlorine, whereas chlorine and sodium hypochlorite are predominantly used in North America, likely due to differences in approved products. The use of surface water in flow-through systems poses a risk of contamination by introducing waterborne fish pathogenic microorganisms, which results in excessive losses in aquaculture globally and has also limited the advancement in commercial farming of new aquaculture species. Bacterial and viral diseases create complications in semi-intensive and intensive aquaculture systems. Before releasing their discharge waters into the aquatic environment, some commercial operations may be needed to disinfect them (Reidun, 2021). Reliable methods of controlling pathogens present in the inlet water are of grave concern (Summerfelt et al., 2003).

2.2.1 Disinfection strategies applied in RAS

The application of chemical treatments in sufficient concentrations and for long enough contact times to kill or inactivate all pathogenic organisms that would otherwise gain access to the surrounding water, persist, and proliferate in the system is emphasised in the general governing principles of aquaculture disinfection (OIE, 2003). RAS disinfection solutions can be categorised as either continuous or periodic. Ozone and UV radiation are used in the full flow of RAS for continuous water disinfection. (Mota et al., 2022) Chemical disinfectants like formalin, copper sulphate, chloramine-T, hydrogen peroxide, and peracetic acid are frequently used for periodic disinfection (Pedersen and Pedersen, 2012).

Disinfection by ozonation or by UV-irradiation or by a combination of both are the most utilised strategies in present day RAS (Summerfelt et al., 2009). It is vital to differentiate between disinfection of makeup waters (low organic loads) and recirculating aquaculture system (RAS) waters (Summerfelt et al, 2003).

2.2.1.1 Disinfection by ozonation and UV irradiation

Both disinfection strategies; ozonation and UV-irradiation, have their pros and cons. Ozone, for example, can be used to oxidise organic molecules and nitrite to improve water quality in addition to inactivating fish pathogens; however, while oxygenating the water, it may leave toxic residues that must be removed before reaching aquatic organisms. Additionally, the production of ozone is costly. (Summerfelt, 2003)

Ozonation can be utilised to enhance water quality in ultra-intensive recirculating production systems. Ozone can create excellent water quality in recirculating systems without resorting to high daily water exchange rates. Ozone can also aid in the reduction of fish disease problems. Ozone has multiple advantages in the aquaculture system for numerous reasons; oxygen is created as a reaction end-product, reaction rate is rapid and very few harmful by-products are produced. The downside is ozone can be dangerous to both fish and humans (Summerfelt et al, 2003). In RAS, ozonation in most cases supports improvement of the physicochemical water quality rather than disinfection (Summerfelt, 2003; Tango and Gagnon, 2003). Ozone is found

unsuitable as a disinfection method in marine RAS for several reasons according to several researchers. For example, a tremendous quantity of ozone is required in general to deactivate bacteria. This is because the oxidative capacity of ozone and residual oxidants are consumed in reactions with organic matter and other components of rearing water. Another example is that several compounds formed when high doses of ozone are added to seawater are highly fatal to fish and live feed (Ozawa et al., 1991; Davis and Arnold, 1997), although running the water through activated carbon filters can help reduce some of the residual oxidants (Kobayashi et al., 1993, Ozawa et al., 1991).

UV radiation is a physical treatment method of water disinfection within the system that can inactivate a wide range of bacteria (Moreno-Andres 2020). UV disinfection is expanding and has gained growing acceptance as a primary disinfection process for water, and it does not produce residual disinfection by-products produced by oxidative disinfectants (Clancy et al., 2000; Linden et al., 2002). A drawback of this system relates to the water quality, as in the RAS water is rich in particulate matter, the outcome of this pattern results in decreased UV penetration and treatment effectiveness (Mamane, 2008).

Compared with ozone, using UV light will not produce toxic residuals or form harmful by-products to fish at all. UV light functions by breaking down the nucleic acids of microorganisms, which will result in death or function loss. Microorganisms can be inactivated at UV wavelengths ranging from 100 to 400 nm, while 254 nm is the most effective wavelength. Ozone residuals can also be removed at specific UV wavelengths from 250-260 nm (Sharrer and Summerfelt, 2007). According to Hunter et al. (1998), complete ozone residual removal can be achieved at UV doses of 60-75 mW s/cm^2, even if the ozone concentration is as high as 0.5 mg/l.

In culture water, the level of ozonation is generally controlled by continuous measurements of the oxidation-reduction potential (ORP). Moderate ozonation to an ORP of about 300–350 mV is normal and considered safe for marine fish in RAS, although some production of toxic bromate has been demonstrated at this level (Tango and Gagnon, 2003). Efficient disinfection in a marine RAS is not achieved by ozonation to 350 mV, however, it has been observed to increase the

physicochemical water quality (Kobayashi et al., 1993, Tango and Gagnon, 2003) resulting in a rise of survival and growth of fish (Ozawa et al., 1991, Reid and Arnold, 1994).

Turbidity, the presence of both organic and inorganic particles in the water, can influence the disinfection effect of UV-irradiation and ozonation (Hess-Erga et al., 2008). UV-transmittance is declined by turbidity and might be blocked by particles, whereas the oxidative power of ozone and residual oxidants can be decreased due to the degradation of disinfectant at the surface of suspended solids and the rate-limited transport into particles (Perrins et al., 2006). In the period, when turbidity is high due to accumulation of small, suspended particles, the effectiveness of disinfection in a RAS is likely to be low for both UV irradiation and ozonation (Reitan et al., 1993; Salvesen et al., 1999). However, the addition of microalgae to the culture water has a beneficial influence on the survival and growth of marine fish larvae during the rotifer period (Salvesen et al., 1999).

Ozonation is a chemical process whereas UV-irradiation is a physical disinfection method. In RAS, the dose and mechanism of these two processes influence stabilisation, maturation, and development of the microbial community over time. In general, RAS are operated at high intensity UV but low or moderate levels of ozonation, the two processes are likely to represent different levels of disinfection efficiency (Attramadal 2012). Ozonation followed by UV irradiation has been applied in wastewater and drinking water treatment to get the best removal of microorganisms for decades (Sharrer and Summerfelt, 2005).

Moreover, numerous studies have demonstrated that water ozonation can effectively reduce chemical oxygen demand (COD), dissolved organic carbon, colour, nitrite nitrogen (NO_2-N), turbidity, total organic carbon, and total suspended solids (TSS) (Good et al, 2017, Summerfelt and Hochheimer, 1997; Tango and Gagnon, 2003; Summerfelt et al., 2009).

2.2.2 Need for Alternate disinfection strategies

Generally, disinfection and antimicrobial effects are achieved by ozonation, and UV irradiation as mentioned before. Despite the benefits, there are several demerits using these systems. Therefore, an alternative water treatment system would be beneficial for the RAS industry.

Peracetic acid-based disinfectants are used on the tanks, as well as the floor, pipelines, and ancillary equipment, and are the most widely used chemicals in Norway, while chlorine is the most widely used chemical in North America, according to a survey by Lazado and Good (2021). In Norway, the three most important factors when choosing a disinfectant were effectiveness against pathogens, user safety, and application ease (Lazado and Good, 2021).

Some other disinfecting compounds like Malachite green has been prohibited for some time (Sudová et al., 2007; Marchand et al., 2012), and the use of formaldehyde is viewed as unfavourable in terms of worker safety and the potential negative environmental impact on the receiving water body (Masters, 2004; Pedersen et al., 2007). However, in vitro tests using PAA have shown that certain products have promising disinfection action (Straus and Meinelt, 2009; Meinelt et al., 2007; Picón-Camacho et al., 2012b). Dose dependent antiparasitic products containing PAA mixtures were used on *Ichthyophthirius multifiliis* (Ich or white spot disease) and critical information on use of PAA in commercial aquaculture operations were discovered (Picón-Camacho et al., 2012a). Although the results have not been clear, PAA were somewhat found efficient on parasites through the in-vivo field trials (Rintamäki-Kinnunen et al., 2005; Sudová et al., 2010). Empirical observations on the effectiveness of PAA-based products against the agent that causes *I. multifiliis* and other troublesome parasites range from fully effective to moderately effective or ineffective preventive measures; farmers have also reported compromised fish health and biofilter impairment (Pedersen and Henriksen, 2011).

2.2.3 Peracetic acid in freshwater RAS; its pros and cons as a disinfectant

Peracetic acid (PAA, CH_3CO_3H), an effective disinfectant used in wastewater treatment facilities and aquaculture (Koivunen and Heinonen-Tanski, 2005), is broken down primarily by chemical oxidation into harmless acetic acid and water, without the production of toxic or harmful by-products (Pedersen et al., 2009). It has been used as an effective disinfectant/sanitiser for certain industrial applications (Davidson et al., 2019). PAA has been described as a powerful oxidant capable of producing water quality benefits comparable to those expected with ozone application (Pedersen et al., 2015); however, the water oxidising capacity of PAA in aquaculture systems and its effects on fish production require further investigation, particularly within RAS. PAA is still in the developing phase as there is still much to learn about their use and potential benefits in RAS. There is a lack of information on how to apply PAA and whether the application of PAA at low concentrations can affect fish welfare (Liu et al., 2017).

In the study presented by Liu et al. (2017), PAA was applied in a pilot-scale carp (*Cyprinus carpio*) production in RAS every 3 or 4 days for 5 weeks. The stress response of fish was monitored during every PAA application. The results from the study summarised that after repeated applications of PAA, carp gradually adapted from its effect. Furthermore, the mathematical model demonstrated that achieving an equal distribution of PAA in RAS was a slow process that was affected by the size and flow of the tank. (Liu et al., 2017)

Mota et al. (2022) reported that the survivability, mucosal health, and swimming behaviour were not affected by the treatment of PAA at concentrations below 1.6 mg/l. Atlantic salmon parr were exposed to nine concentration doses of PAA ranging from 0 - 6.4 mg/l. However, damaged skin, gill necrosis and mortality were observed with higher PAA concentration doses. The survival rate of the salmon parr was 100% when the fish were exposed to PAA concentration between 0 to 1.6 mg/l, whereas PAA concentration of 3.2 mg/l and 6.4 mg/l resulted in 80% and 0% survival rates respectively within an hour of exposure. Furthermore, the study reported that the responses of salmon parr and smolts to PAA concentrations differ extensively. (Mota et al., 2022)

The investigation of physiological impacts and structural changes from the application of PAA in Atlantic salmon parr in freshwater RAS was performed and reported by Carletto et al. (2022). PAA of concentration of 1 mg/l was administered in the same amount via two modes (1) continuously; in every 3 hrs and (2) pulse every 3 days, over a duration of 4 weeks. The influence on the physiological and structural changes were reported to be predominantly affected by the time duration of the trial than from the mode of PAA administration. (Carletto et al., 2022)

Pedersen et al. (2009) advised bypassing the biofilter when using PAA in RAS because PAA as low as 2 mg/l can harm the nitrifying microorganisms in the biofilter (Liu et al., 2017). However, PAA has been shown to be effective against a variety of fish pathogens at low concentrations (Pedersen et al., 2013). Water quality improvement has not been observed by the application of PAA as compared to low dose ozone application. PAA dosing at concentrations of 0.05 - 0.30 mg/l was compatible with trout production and biofilter performance (Liu et al., 2017). Oxidative reduction potential (ORP) increased with PAA concentration indicating the potential for ORP to control PAA residuals. Large doses of PAA (2.8 - 9.3 mg/l) are known to induce lethal effects on fish (Straus et al., 2018; Lindholm, 2022).

3. Materials and Methods

3.1 Experimental design

3.1.1 Samples and sample preparation

Stored RAS tank water samples from an experimental trial (October – December 2022) at Havbruksstasjonen i Tromsø, Kårvika were used as the main sample for the experiments. The samples were transferred to the NMBU water lab in a frozen state in two 20-litre Jerry cans. The water sample which is mentioned as 'PAA - adapted' throughout the document was procured from Tank number 7 of the trial which was exposed to prior 4-weeks PAA treatment during the trial. Whereas the water sample which is mentioned as 'PAA - naive' throughout the document was procured from Tank number 8 of the trial which was one of the control tanks. The water quality parameters like COD, TSS, and turbidity were analysed on both PAA - adapted and PAA – naive water samples in the beginning in order to prepare samples with different feed loads with complimentary water quality parameters in the laboratory. Samples with seven levels of feed loads with the concentration of 10 mg/l, 15 mg/l, 20 mg/l, 30 mg/l, 40 mg/l, 50 mg/l, and 80 mg/l were prepared by pulverising and dissolving standard Salmon feed in deionised water. The feed used for the sample preparation was Nutra RC 3.0 mm standard salmon feed from Skretting AS, Norway, which was also obtained from Trømso. The feed was ground to a powder using a mortar and a pestle and weighed using the balance to prepare a stock solution. A magnetic stirrer was used to mix and homogenise the feed solution properly. The stock solution was diluted to the desired concentrations. The feed solutions were subjected to similar experiments and conditions as that of the RAS tank water samples to observe and compare the consumption of PAA. All the samples were prepared as identical triplicates and the triplicates were treated and analysed under identical conditions to increase the reliability and the statistical significance of the data obtained. In addition, one blank each for every sample replicate was prepared using DI water and treated and analysed under identical conditions to that of the samples in order to assess the

validity of the protocol and reduce measurement errors. The between triplicate variation was assessed by calculating the sample standard deviation.

3.1.2 Set-up

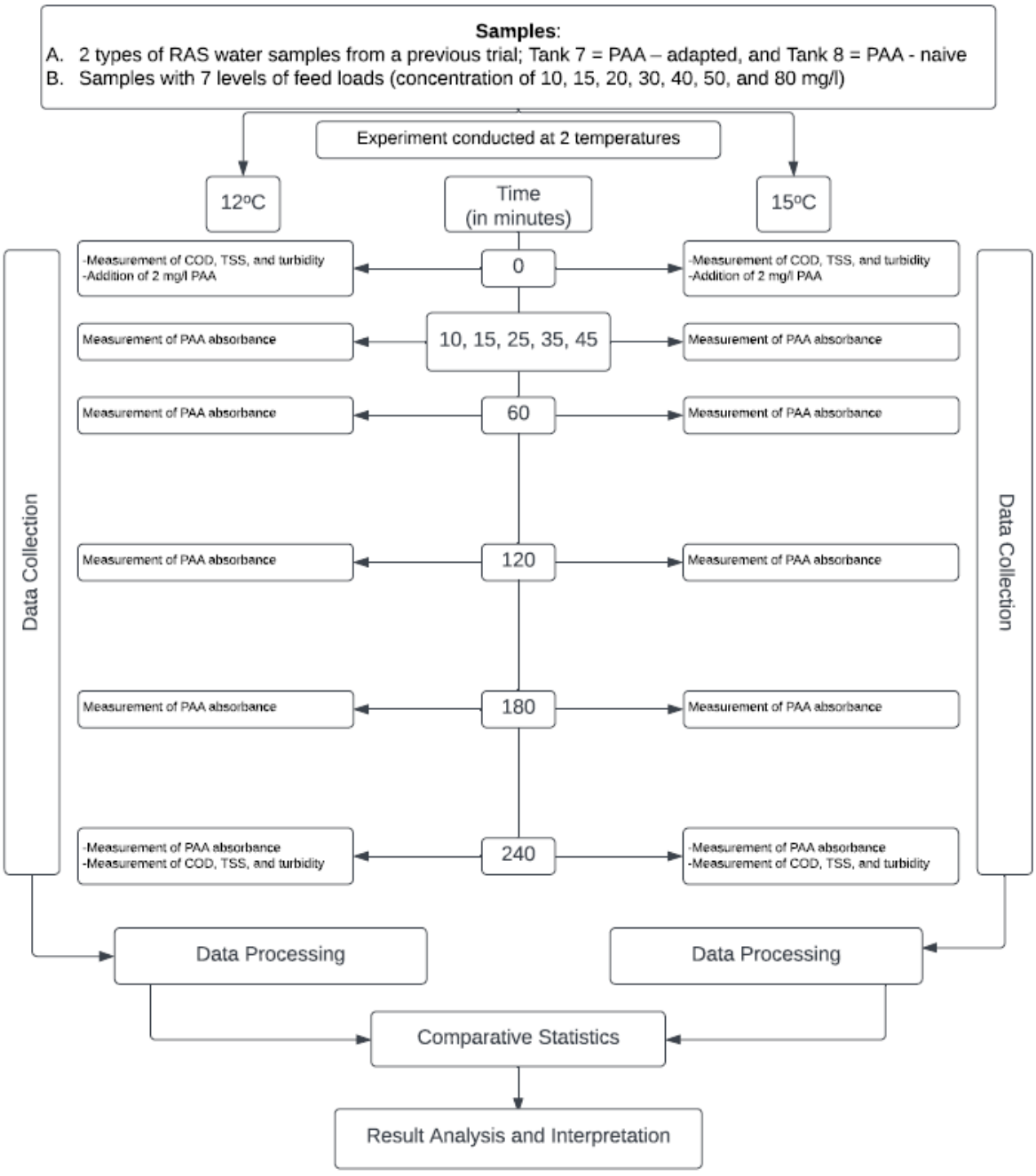

Figure 2. Summary of the experimental design (Made with Lucidchart https://lucid.app/)

All the samples were tested following similar protocols and conditions at two different temperatures: 12°C and 15°C. These temperature points were chosen because of their significance in Atlantic salmon production in freshwater RAS. PAA treatment in the concentration of 2 mg/l was applied on all the water samples and the concentration was monitored over the course of four hours to understand the decay of PAA in all the samples. The raw absorbance data obtained from the Spectrophotometer was utilised to calculate the concentration of PAA over time. These concentrations were plotted in a scatter plot and a logarithmic function was fitted to them to get the slopes. The slopes were utilised for half-life calculations and for the descriptive statistics to interpret the results with a statistical significance. The water quality parameters were tested at time 't_0' = 0 minute and time 't_1'= 240 minutes to quantify changes in the same due to 240 minutes of PAA treatment at 12°C and 15°C respectively. A summary of the experimental design used for the formulation of this document is illustrated in Figure 2.

3.2 Analytical Grade Chemicals

Peracetic acid 15% was purchased from Aqua Des, Aquatic Chemistry AS, Lillehammer, Norway in a 22 kg container. Ethylenediaminetetraacetic acid Dihydrate (EDTA.2$H_2$0, E1) and Potassium iodide (KI) (K9) were purchased from Merck Life Science AS/Sigma Aldrich Norway AS, Filipstad Brygge 1, 0252 Oslo, Norway. Concentrated sulphuric acid 95-97% for analysis EMSURE® ISO (96% H_2SO_4) was purchased from Merck KGaA, D-64271, Darmstadt, Germany. N, N-diethyl-p-phenylenediamine sulphate salt (DPD salt, D25) and Sodium phosphate dibasic heptahydrate for analysis EMSURE® ACS ($Na_2HPO_4.7H_2O$) (D25) were purchased from Merck Life Science AS Drammensveien 123, 5th floor, N-0277 Oslo, Norway. Potassium dihydrogen phosphate (KH_2PO_4) was purchased from VWR International AS, Haavard Martinsens vei 30, 0978 Oslo, Norway.

3.3 Other Materials

Deionised Water from the DI system in the laboratory in TF Fløy V, NMBU was used for the preparation of the reagents, stock solutions and samples and for all the experimentations.

Spectroquant® COD Cell Tests 4.0 - 40.0 mg/l and 0.7 µm binder-free Whatman glass fibre filter paper (47 mm diameter) (class 698 in VWR) was purchased from VWR International AS, Norway.

Borosilicate glass volumetric flask grade A from VWR International AS was used for measuring the volumes of all liquids. The weighing scale from VWR with a minimum limit of 20 mg was used for weighing all the materials and samples. The automatic pipettes of 1000 µl, 5 ml and 10 ml with their respective pipette tips that were used for the experiments were from VWR.

3.4 Measurement of water quality parameters

In addition to the measurement of the concentration of PAA over the course of 240 minutes, three other water quality parameters; TSS, turbidity, and COD, were measured to compare the changes in the same parameters in the sample before and after 240 minutes of treatment with 2 mg/l PAA.

3.4.1 Measurement of PAA

The experiments were conducted at two different temperatures; 12°C and 15°C to observe the temperature-dependent changes in the water quality parameters and the consumption of PAA. These temperatures were chosen because of their relevance to the salmon industry as smolt production in Atlantic salmon in RAS farms is usually between 8°C and 14°C. Termaks Series 6000 cooling incubator with the range from 0°C to 70°C was used to conduct all the experiments requiring 12°C temperatures. The experimentations at 15°C were conducted on the lab bench because the room temperature was approximately 15°C according to the thermostat.

3.4.1.1 Preparation of PAA stock solution

The stock solution was prepared fresh for each day in 1-litre volume and 1000 mg/l concentration. The volume of PAA solution required to achieve the desired concentration was calculated using the formula below.

$$C1V1 = C2V2$$

Where,

C1 = Initial concentration of the acid from the container

V1 = Volume of acid required to reach the desired concentration

C2 = Final desired concentration of the stock solution

V2 = Prepared volume of the stock solution.

1 litre of deionised (DI) water was measured using a volumetric flask and poured into a borosilicate glass bottle with a cap. 6.67 ml of the DI water was pipetted out of the bottle and replaced with 6.67 ml of Peracetic acid 15%. The solution was mixed thoroughly before use.

3.4.1.2 Chemical analysis to measure PAA concentration

PAA-DPD Method after Santoro / Dell'Erba + Falsanwasi, modified from DTU AQUA was used to quantify the decay of PAA over time. N, N-diethyl-p-phenylenediamine (DPD) reacts with peracetic acid at pH 6.5 to give a red colour complex (DPD · +). The reaction was catalysed with KI and measured spectrophotometrically at 550 nm.

3.4.1.2.1 Preparation of reagents

Two reagents are required to measure the concentration of Peracetic acid using the PAA-DPD method which will be mentioned throughout the document as R1 and R2

Preparation of Reagent 1 (R1 = N, N-diethyl-p-phenylenediamine sulphate salt (DPD) 1.6%)

0.1274 g of Ethylenediaminetetraacetic acid Dihydrate (EDTA.2H_2O, E1) and 1 ml of 96% H_2SO_4 were added to 250 ml of DI water. Then 8 g of N, N-diethyl-p-phenylenediamine sulphate salt (DPD salt, D25) was added to the mixture. The solution was made to 500 ml volume by the addition of DI water. The reagent was stored in a dark bottle and left until a pale pink colour appeared.

Preparation of Reagent 2 (R2 = Buffer solution of pH 6.5 with KI)

22.64 g of $Na_2HPO_4.7H_2O$ (D25), 23 g of KH_2PO_4 (K4) and 0.5 g of KI (K9) were dissolved in a beaker with 400 ml DI water and the pH was adjusted to 6.5. Then the solution was transferred to a 500 ml volumetric flask and made up to the mark with DI water.

The buffer reagent was stored in a dark bottle. It is stable for at least 3 months if stored properly in the dark.

3.4.1.2.2 Procedure for measurement of PAA

2.5 ml of the sample was pipetted into a clean glass cuvette to which 0.25 ml (250 µl) of DPD reagent (R1) was added and mixed. Then 0.25 ml (250 µl) of KI buffer at pH 6.5 (R2) was added. A timer of 30 seconds was immediately started after the addition of R2. The lid on the glass cuvette was closed and the mixture was shaken, and the sample was measured at 550 nm using a UV/VIS spectrophotometer (model: UV-T500PRO). The reading at the 30-second mark was taken to calculate the concentration of PAA. Each time, the side with the G marking was faced towards the source of light in a spectrophotometer and the cuvette was placed exactly on the middle to minimise reading errors. A summary of the procedure is illustrated in Figure 3. The glass cuvette was rinsed with DI water thrice and then with the sample between samples.

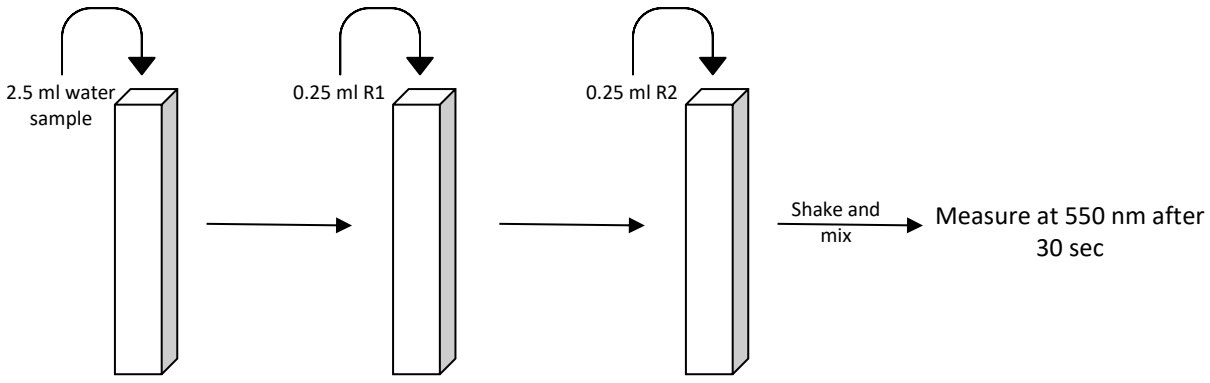

Figure 3. Procedure for the measurement of PAA using the PAA-DPD method.

3.4.1.2.3 PAA standard curve

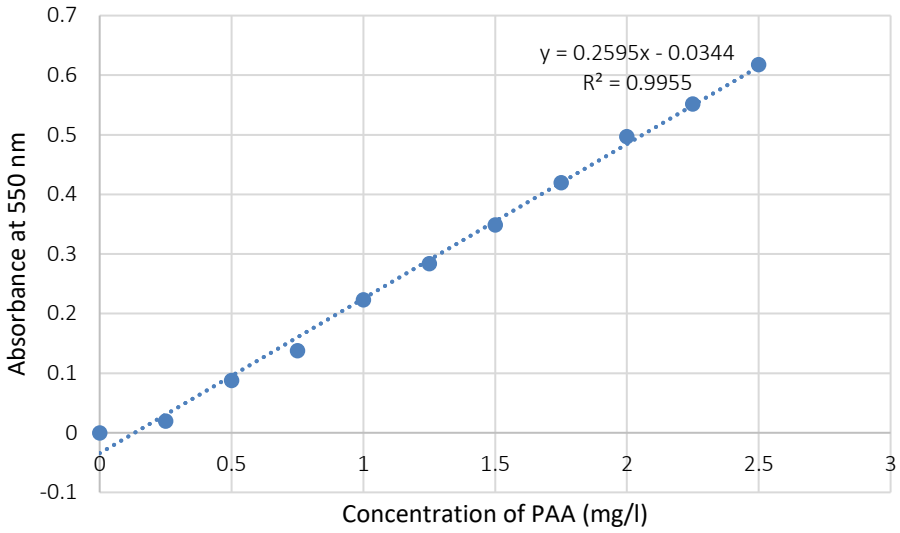

Figure 4. Standard curve of PAA absorbance

The standard curve for PAA was constructed to derive the standard formula for calculating the concentration of PAA from the absorbance value. PAA standards of 0 mg/l, 0.25 mg/l, 0.50 mg/l, 0.75 mg/l, 1.00 mg/l, 1.25 mg/l, 1.50 mg/l, 1.75 mg/l, 2.00 mg/l, 2.25 mg/l, and 2.50 mg/l were prepared. The same formula as for the PAA stock solution was used to prepare the standard dilutions. The absorbance of PAA in the standards was measured using the PAA - DPD Method and the values were plotted in a graph illustrated in Figure 4. Using a linear regression model, the linear equation and the R-squared values were determined; this equation was utilised to calculate the concentration of PAA in further experimentation.

3.4.1.2.4 Preparation of samples and PAA measurement

500 ml of the sample volume was measured and transferred to a 500 ml BOD glass bottle. Then a magnetic stirrer was added to each of the bottles. WTS OxiTop® IS 6 Inductive Stirring System (Germany) was used to mount the glass bottles with the samples and the blanks to apply continuous stirring. At time = 0 minutes, i.e., before the addition of PAA into the water sample, sample volumes were taken to conduct TSS, turbidity and COD test. The volume of 1000 mg/l

PAA stock solution required to reach the desired concentration of 2 mg/l in the water samples was calculated and then added. The timer was immediately started after the addition of PAA. Blanks with DI water were prepared following the same procedure and under similar conditions as that of samples. The absorbance of PAA in the blanks and the samples were measured and noted at 10, 15, 25, 35, 45, 60, 120, 180 and 240 minutes. These values were used to calculate the concentration of PAA in the respective samples and blanks at the respective times using the standard equation derived from the PAA standard curve. At time = 240 minutes, i.e., after 4 hours of PAA treatment of the water samples, sample volumes were taken to conduct TSS, turbidity and COD test. A model of the experimental set-up used in the laboratory for the measurement of PAA is illustrated in Figure 5.

Figure 5. Experimental set-up for the measurement of PAA in water samples using a Spectrophotometer.

3.4.2 Measurement of Total Suspended Solids

The standard protocol NS-EN 872:2005 was followed to determine the TSS of the water samples. This European Standard describes the method for the determination of suspended solids in raw waters, waste waters and effluents by filtration through glass fibre filters with a lower limit of the determination at about 2 mg/l. The filtration set-up was equipped with a Diaphragm vacuum pump with an ultimate vacuum pressure of 50 Mbar. A single unit of Whatman GF filter paper was placed in labelled aluminium trays and put in the drying oven (Termaks) at 105°C for at least 20 minutes before the experiment. The filter papers were weighed before use and the individual weight was noted. The filter paper was placed smooth side down on the funnel of the filtration

set-up and the device was connected to the vacuum. The samples were pre-measured to a set volume. The bottles with the samples were shaken and the sample was transferred onto the filtration setup. About 20 ml of DI water was taken to rinse the sample bottle and this was also transferred for filtration. After the sample was completely filtered, another 20 ml of DI water approximately was taken to rinse the insides of the funnel. When the filter was almost dry, the vacuum was released, and the filter paper was gently transferred to the aluminium tray with the help of clean forceps with flat ends.

The filter paper was dried in the drying oven at 105±2°C for at least an hour. After removing from the oven, the filter paper was left to attain equilibrium with the surrounding air near the balance and it was weighed as before. Figure 6 shows a model of the experimental set-up used in the laboratory to determine the TSS of the water samples.

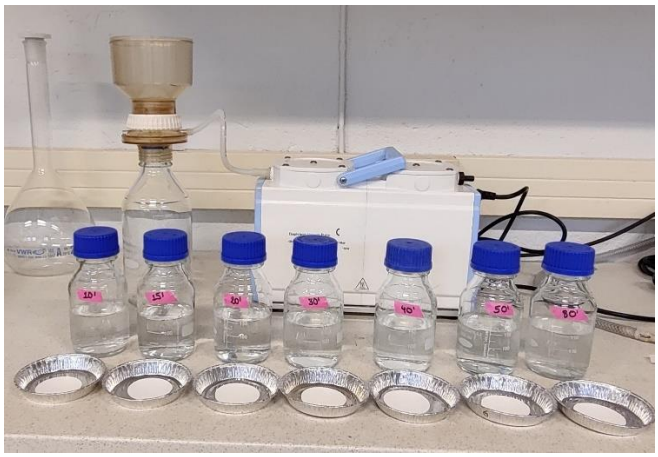

Figure 6. Filtration set-up with a vacuum pump to determine the TSS of the water samples.

The following formula was used to determine the content of total suspended solids in a sample.

$$TSS = [1000 * (B - A)]/V$$

Where,

B = mass of the filter paper after filtration in milligrams

A = mass of the filter paper before filtration in milligrams

V = Volume of sample in millilitres

3.4.3 Measurement of Turbidity

The turbidity of all the samples were measured using HACH® 2100Qis Portable Turbidimeter. Turbidity was measured with protocol compliance with the standard NS-EN ISO 7027-1:2016 for the measurement of turbidity. The portable turbidimeter comes with calibration standards of 10 NTU, 20 NTU, 100 NTU and 800 NTU. The metre was calibrated following the device instructions each time before measuring the samples. 15 ml of sample was pipetted out to a clean glass vial that comes with the turbidimeter, the cap of the vial was closed, and the outer surface was polished with Kinmac paper. The vial was placed in the sampler in the metre, and the lid was clicked shut before pressing the ¨read¨ button to minimise the loss of radiation. The reading displayed on the screen in FNU was noted. The glass vial was washed three times with DI water and once with the respective sample between samples.

3.4.4 Measurement of Chemical Oxygen Demand

The Spectroquant® COD Cell Test was used to measure the chemical oxygen demand (COD) in the water samples. The COD thermoreactor or the heating block (QBH2, Grant, Grant instruments, England) was switched on inside a fume hood and the temperature was set to 148°C. COD cell tests were put in a test tube rack and labelled with the sample ID. The bottom sediment in the reaction cells was suspended by swirling. 3 ml of sample was pipetted and carefully allowed to run down from the inside of the tilted reaction cell onto the reagent. The screw cap to the cell was tightly closed.

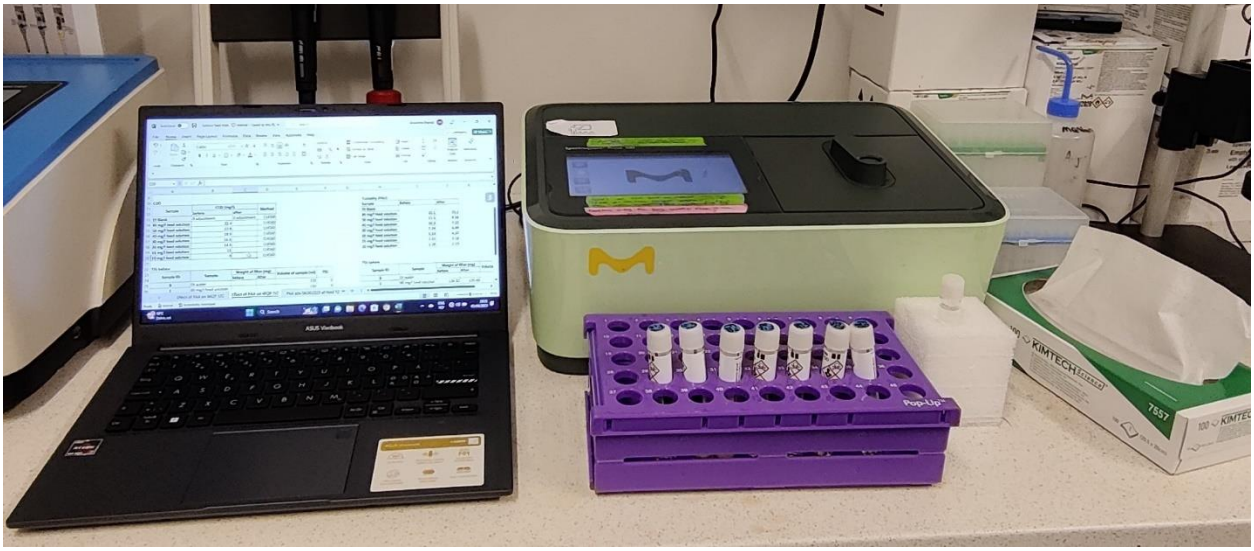

Figure 7. Set-up for the measurement of COD with Merck Spectroquant Prove 100

In all subsequent steps, the cell was held only by the screw cap because the reaction generated an abundant amount of heat making the cell very hot. The contents of the cell were vigorously mixed and then the cell was placed in the preheated thermoreactor at 148°C for 120 minutes. After 120 minutes, the hot cells were removed from the thermoreactor and allowed to cool in a test-tube rack. Ten minutes after placing it in the rack, the cells were mixed. The cell was allowed to rest and cool to room temperature for at least 30 minutes before measuring the COD using a Merck Spectroquant Prove 100 (illustrated in Figure 7). The Zero adjustment was done using a zero cell with DI water by choosing the desired method in the Spectroquant before measuring the samples. The methodology as provided by the supplier is attached in the Appendix *1*. For the samples with estimated COD values higher than the range of the cell test used, a 50% dilution was done using DI water and the observed COD values were subsequently multiplied by 2.

3.5 Calculations and statistical analysis

The findings are presented as an average (with a sample standard deviation to determine between replicate error) based on three replicates. Microsoft Excel was exclusively used for compiling and modelling the data. For the analysis of PAA decay, the scatter plots were prepared by the concentration data of PAA derived from the absorbance data noted from the

spectrophotometer. A Goodness of fit analysis and the R-squared values were utilised to choose the line of the best fit. PAA and H_2O_2 degradation exhibit exponential decay (Newman 1995). The first-order decay rate constant (k, h-1) is consequently computed using the formula:

$$C_t = C_0 * e^{-kt}$$

where C_t is the concentration of PAA or H_2O_2 at time t (h), and C_0 denotes the initial concentration, k is the decay constant, and e is the mathematical constant approximately equal to 2.71828 (Pedersen and Lazado, 2020). k is determined as the regression coefficient of the ln-transformed concentration values versus time using the same set of data, or it is deduced from the exponential regression analysis using only concentrations above 0.1 mg/l (Pedersen and Lazado, 2020).

There are two types of mathematical functions that can be used to model decay: exponential and logarithmic. Due to the particular characteristics of the data, such as the range of values or the existence of outliers, a logarithmic curve might in certain instances fit an exponential decay data plot more accurately. The model's quality of fit must be examined, and its ability to correctly forecast values must be evaluated. Even if the component exhibits exponential decay, using a logarithmic function to describe the decay of the component may be justified provided the model fits the data well and correctly forecasts the values. (Sokal and Rohlf, 1987)

Based on the Goodness of fit test and the R-squared values, it was observed that the logarithmic function fits slightly better than the exponential function on the PAA decay data obtained for this report. The slope of the logarithmic function was utilised to calculate the Half-life and to conduct further statistical analyses. The logarithmic function is in the format.

$$y = a * Ln(x) + b$$

Where the slope is represented by the coefficient 'a' that multiplies the natural logarithm of 'x'.

The formula for the half-life ($T_{1/2}$) is.

$$T_{1/2} = \ln(2) / k$$

where $T_{1/2}$ is the half-life of the substance, ln (2) is the natural logarithm of 2 (which is approximately 0.693), and k is the rate constant of the decay reaction.

Both Microsoft Excel version 2304 and IBM SPSS Statistics version 29.0.1.0 were used to conduct the analyses. Two-way ANOVA was used for the statistical analyses, and a difference was deemed statistically significant if $p \leq 0.05$. To ensure that the assumptions of the two-way ANOVA were met, the data was subjected to tests for normality, homogeneity of variance, independence, and factorial independence. Normality was checked using the Shapiro-Wilk test, and homogeneity of variance was checked using Levene's test. Independence was ensured by examining the study design, and factorial independence was checked by examining the interaction term in the ANOVA output.

4. Results

4.1 Comparison in the consumption of PAA in an adapted and a naive RAS water samples at two temperatures

A comparison of the consumption of peracetic acid (PAA) between the RAS water samples from PAA-adapted and a PAA-naive systems at two temperatures; 12°C and 15°C, showed that PAA decayed at a higher pace and to a much lower concentration in the adapted water samples. Figure 8 illustrates the scatter plot of the mean concentration of PAA in each sample system along with the sample blanks at different temperatures.

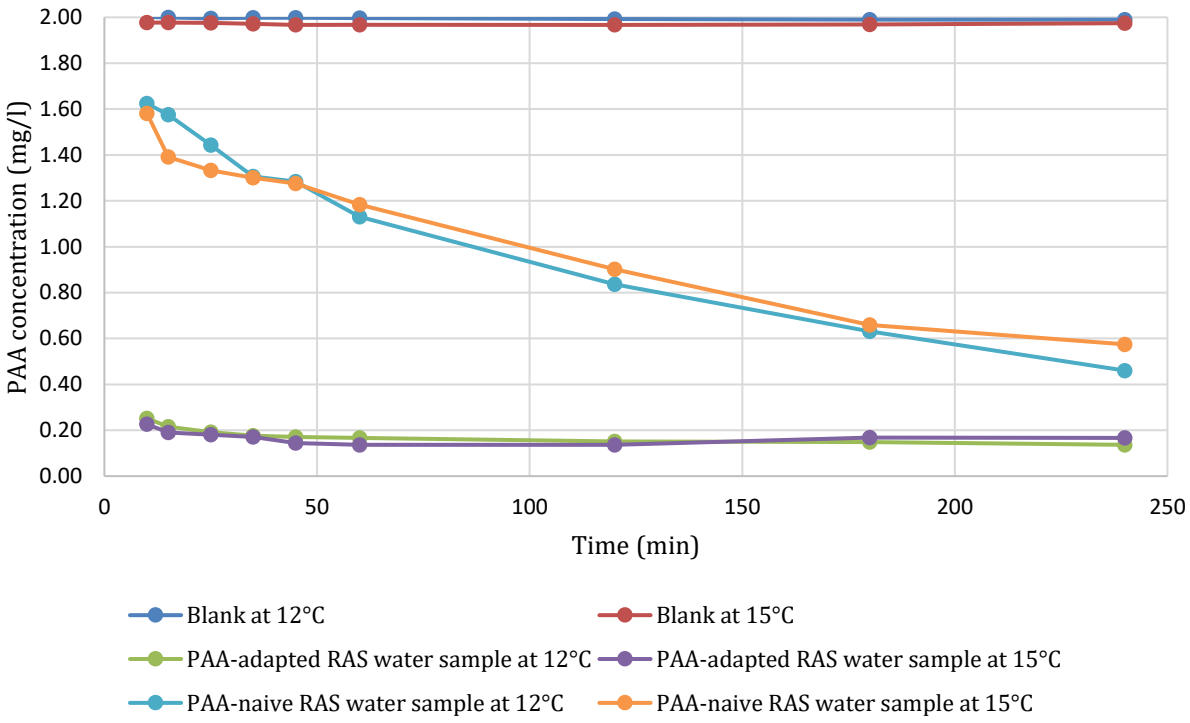

Figure 8. Comparison of PAA decay in an adapted and a naive RAS water samples at 12°C and 15°C. The plots are an average of three replicates of each sample and blank.

The consumption of PAA in an adapted system was very rapid at the start for both treatment temperatures, with more than approximately 87% reduction from the initially added peracetic acid. Samples from the PAA-naive system showed a more gradual and exponential decay of the compound. The difference in the decay rate of PAA in the adapted and the naive system was remarkably high and was deemed highly significant ($p < 0.001$) based on the results of two-way ANOVA analysis. In addition, it also indicated that temperature as a variable only slightly impacts PAA consumption in both sample systems, with slightly higher PAA consumption at the beginning of the treatment observed at higher temperatures.

4.2 Comparison in the consumption of PAA in samples with different feed loads at two temperatures

The average consumption of PAA in seven different levels of feed solution, over the course of 240 minutes at 12°C and 15°C, are plotted in Figure 9 and Figure 10 respectively. It was observed that PAA decay was higher at 15°C than at 12°C in samples with higher feed loads (30 mg/l, 40 mg/l, 50 mg/l, and 80 mg/l). In all the feed loads assessed, the decay was rapid at 15°C at the beginning of the treatment. The two-way ANOVA analysis indicated that that the model is significant (p-value < 0.001), indicating that there is a significant effect of the independent variables - temperature and feed load on the dependent variable - PAA consumption.

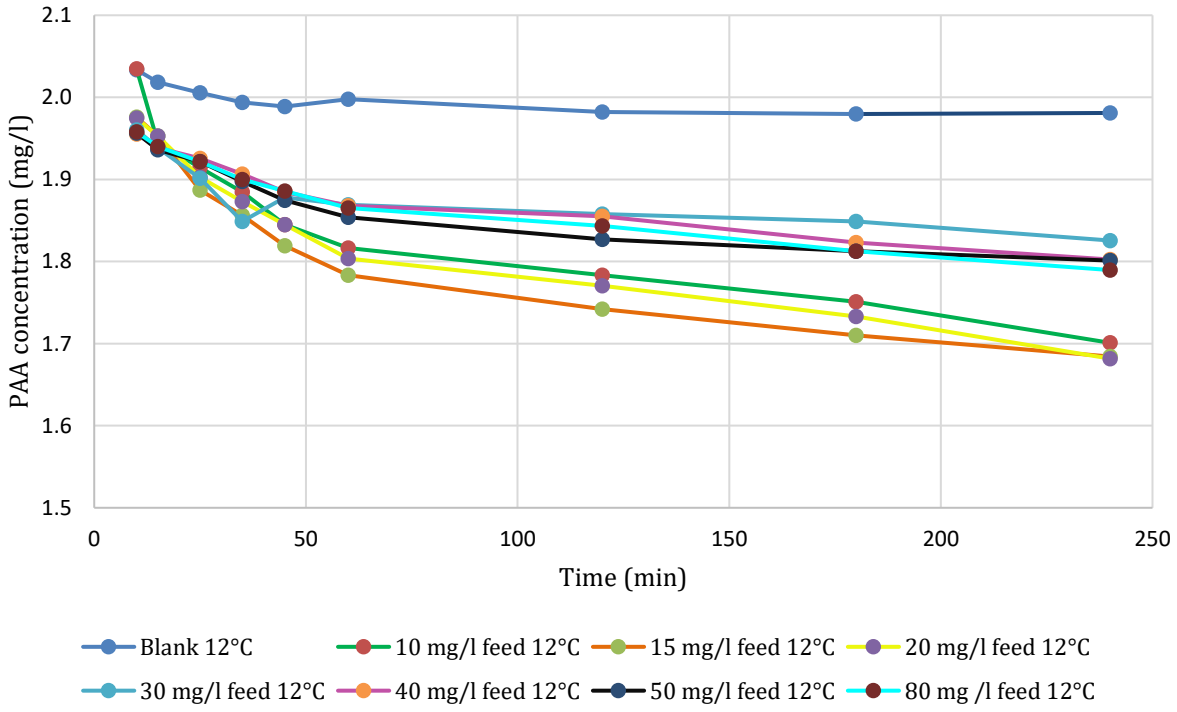

Figure 9. PAA concentration in all feed levels at 12°C. The plots are an average of three replicates of each sample and blank.

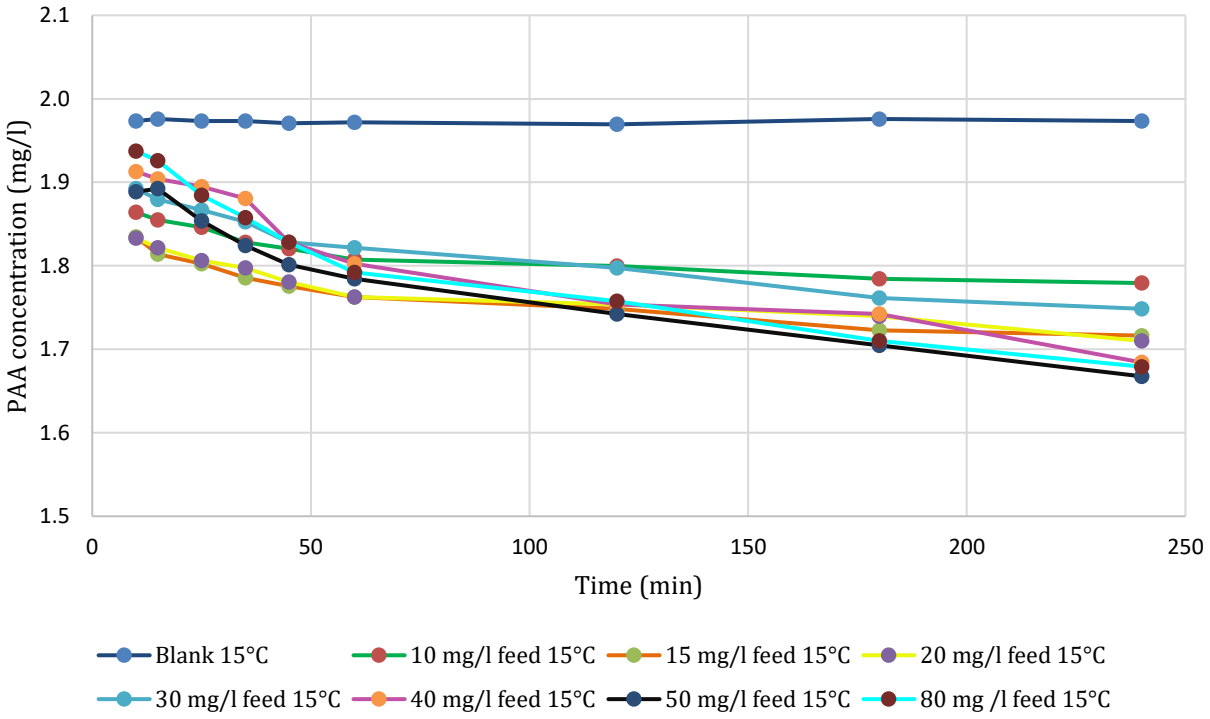

Figure 10. PAA concentration in all feed levels at 15°C. The plots are an average of three replicates of each sample and blank.

4.3 Changes in water quality parameters as a result of PAA treatment at two temperatures

4.4.1 PAA-adapted samples

The COD after 240 minutes of 2 mg/l PAA treatment was higher than the COD before treatment for the PAA-adapted water samples (summarised in Figure 11). The COD increased by an average of 11.70% at 12˚C and by an average of 16.80% at 15°C. The results of a between-subjects ANOVA with the dependent variable of COD and the independent variables of Temperature and PAA treatment indicates that the model is statistically significant (p-value < 0.001). However, the interaction effect between Temperature and PAA treatment was not statistically significant (p-value > 0.05). This implies that both Temperature and PAA treatment had a significant main effect

on COD; with higher treatment temperature resulting in a larger increase in COD in the sample, but there is no significant interaction between the two.

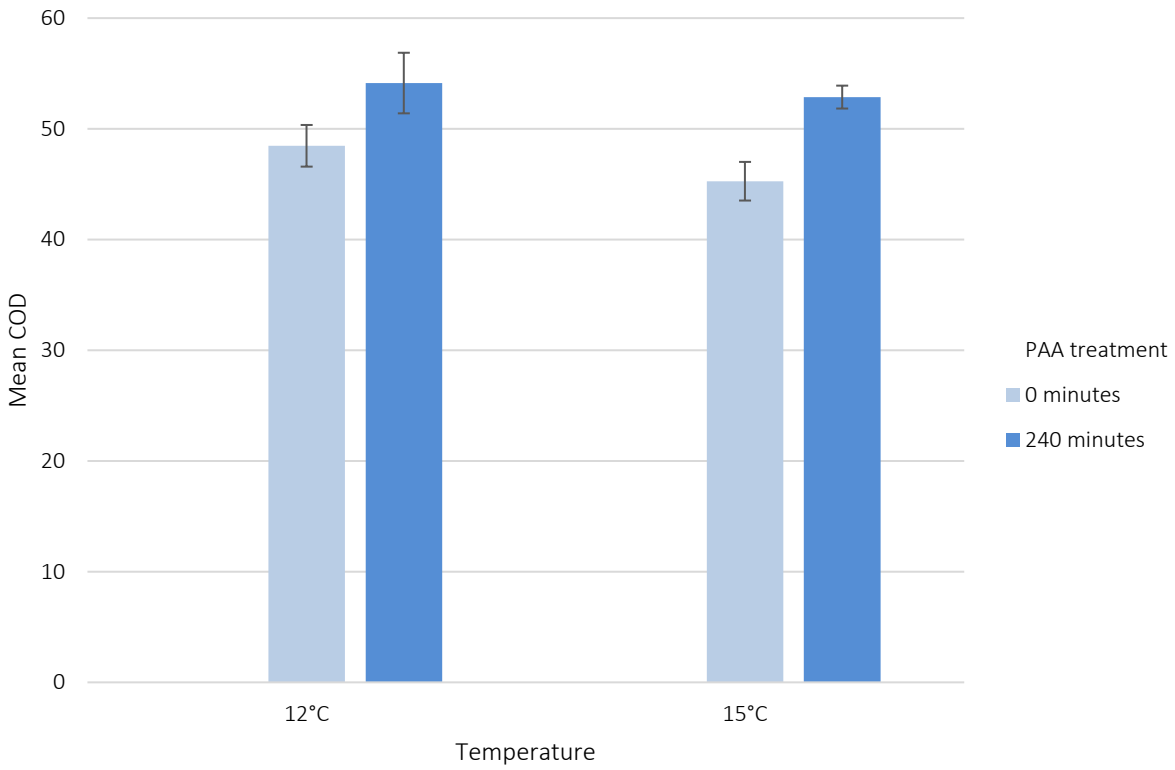

Figure 11. Averages of COD from triplicates of PAA-adapted water samples at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C and 15°C

The TSS of the PAA-adapted water samples decreased after 240 minutes of 2 mg/l PAA treatment by an average of 16.48% at 12°C and an average of 14% at 15°C (summarised in the bar chart in Figure 12). Upon statistical analysis by two-way ANOVA, it was observed that there was a significant main effect of Temperature and PAA treatment on the change in TSS ($p < 0.001$).

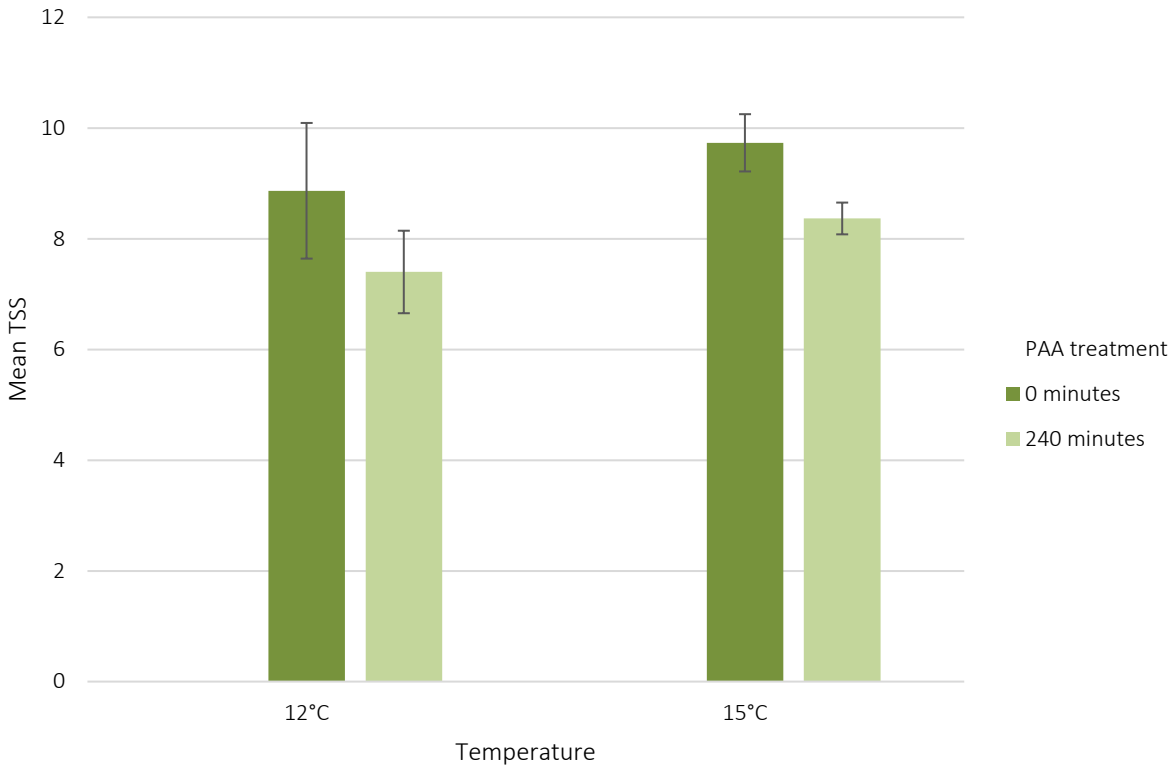

Figure 12. Averages of TSS from triplicates of PAA-adapted water samples at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C and 15°C

There was a significant decrease (with p-values < 0.001) in the turbidity of the PAA-adapted water samples after 240 minutes of 2 mg/l PAA treatment (Figure 13). The turbidity decreased by an average of 4.71% at 12°C and by an average of 7.03% at 15°C. The results of two-way ANOVA also suggested that the decrease in turbidity was significantly higher at the higher temperature. The partial eta squared values indicated that the main effect of Temperature and PAA treatment explained the most variance in Turbidity (85.3%).

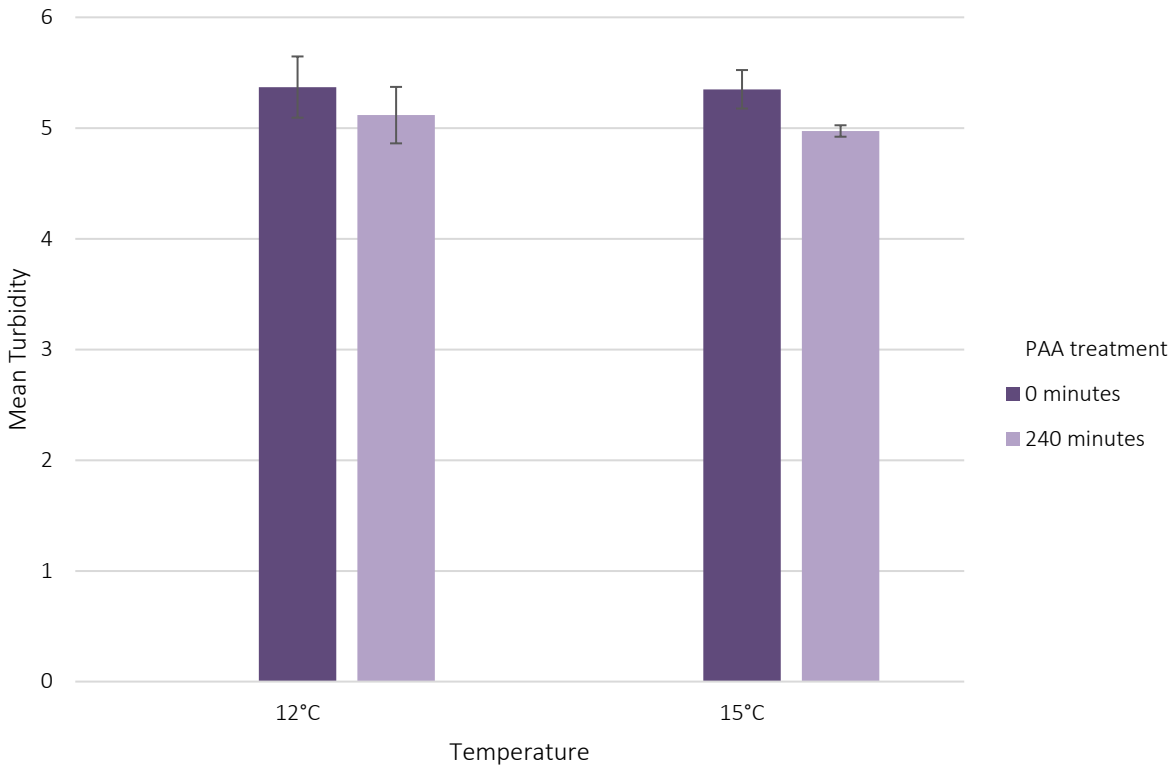

Figure 13. Averages of turbidity from triplicates of PAA-adapted water samples at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C and 15°C

4.4.2 PAA-naive RAS water samples

The COD of the PAA-naive RAS water samples increased after 240 minutes of 2 mg/l PAA treatment by an average of 10.44% at 12°C and by an average of 11.01% at 15°C. The mean COD of the triplicate samples of the PAA – naive RAS water before and after PAA treatment at 12°C and 15°C are summarised in Figure 14. The results of a between-subjects ANOVA with the dependent variable of COD and the independent variables of Temperature and PAA treatment indicated that the overall model is significant ($p < 0.001$), which means that the independent variables as a group have a significant effect on the dependent variable. The results also showed the main effect of the intercept, which is significant ($p < 0.001$). However, the interaction effect between Temperature and PAA treatment is not significant ($p > 0.05$). This implies that although the COD increases as a result of PAA treatment over time, there might not be much effect on this increase due to the treatment temperatures.

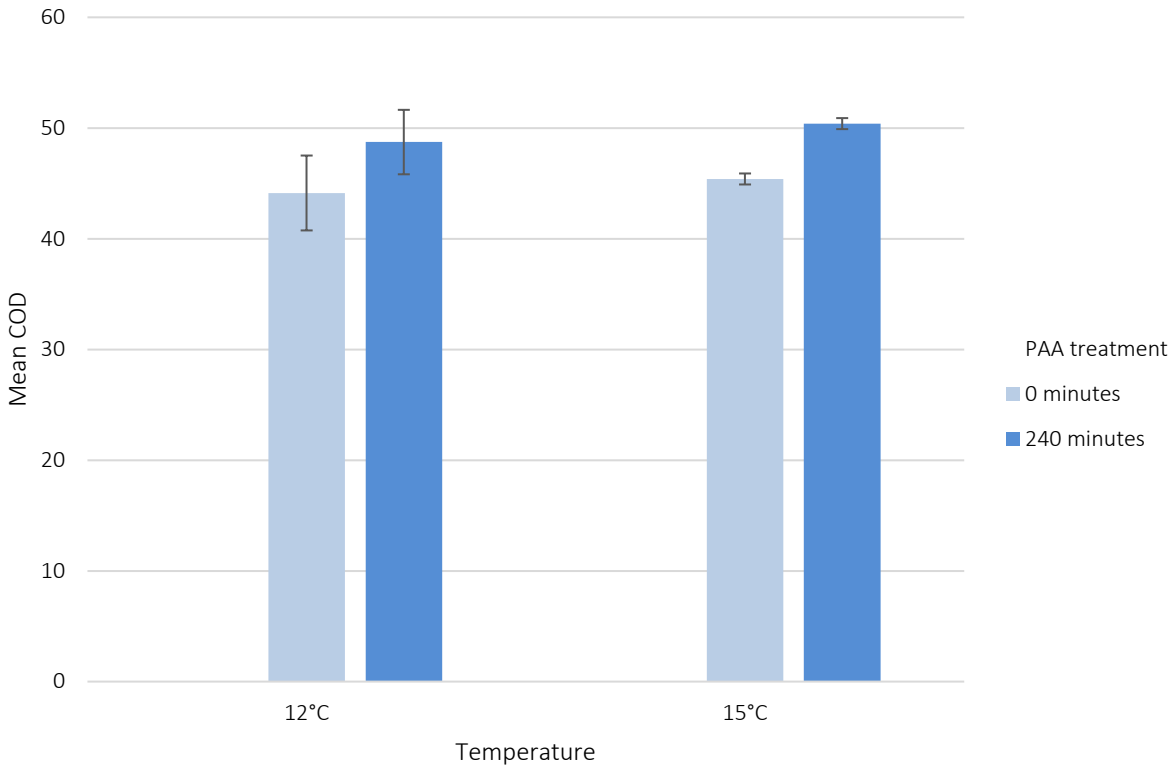

Figure 14. Averages of COD from triplicates of PAA-naive water samples at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C and 15°C

The average TSS of the triplicate samples of the PAA – naive RAS water after PAA treatment at 12°C and at 15°C are summarised in Figure 15. The TSS of the PAA-naive water samples decreased after 240 minutes of 2 mg/l PAA treatment by an average of 10.08 % at 12°C and an average of 26% at 15°C. The results of the statistical analysis by two-way ANOVA indicated that the Corrected Model (including the intercept and the interaction between Temperature and PAA treatment) is significant ($p < 0.05$), which means that the model explains a significant amount of variance in TSS.

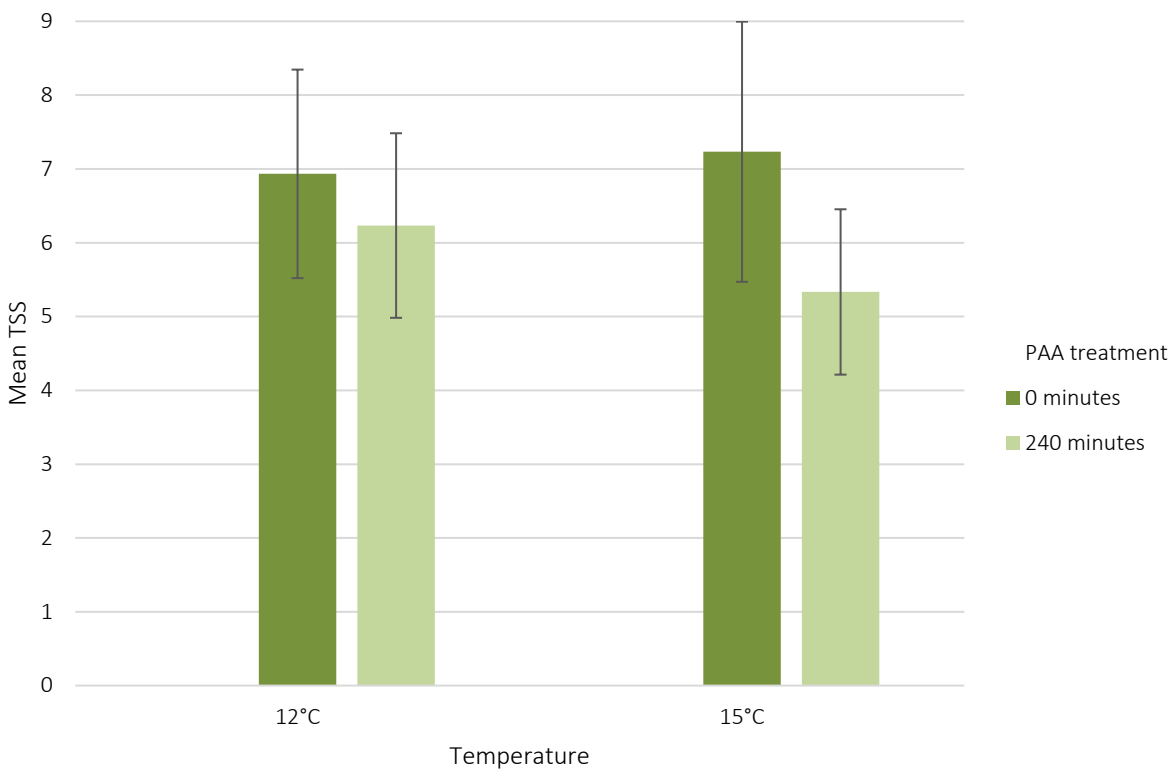

Figure 15. Averages of TSS from triplicates of PAA-naive water samples at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C and 15°C

The average turbidity of the triplicate samples of the PAA – naive RAS water after PAA treatment at 12°C and at 15°C are summarised in Figure 16. There was a significant decrease (with $p < 0.001$) in the turbidity of PAA - naive water samples after 240 minutes of 2 mg/l PAA treatment. The turbidity decreased by an average of 2.20% at 12°C and by an average of 4.14% at 15°C. According to the result from 2-way ANOVA, the corrected model row indicated that the model as a whole account for a significant amount of variation in the dependent variable ($p < 0.001$).

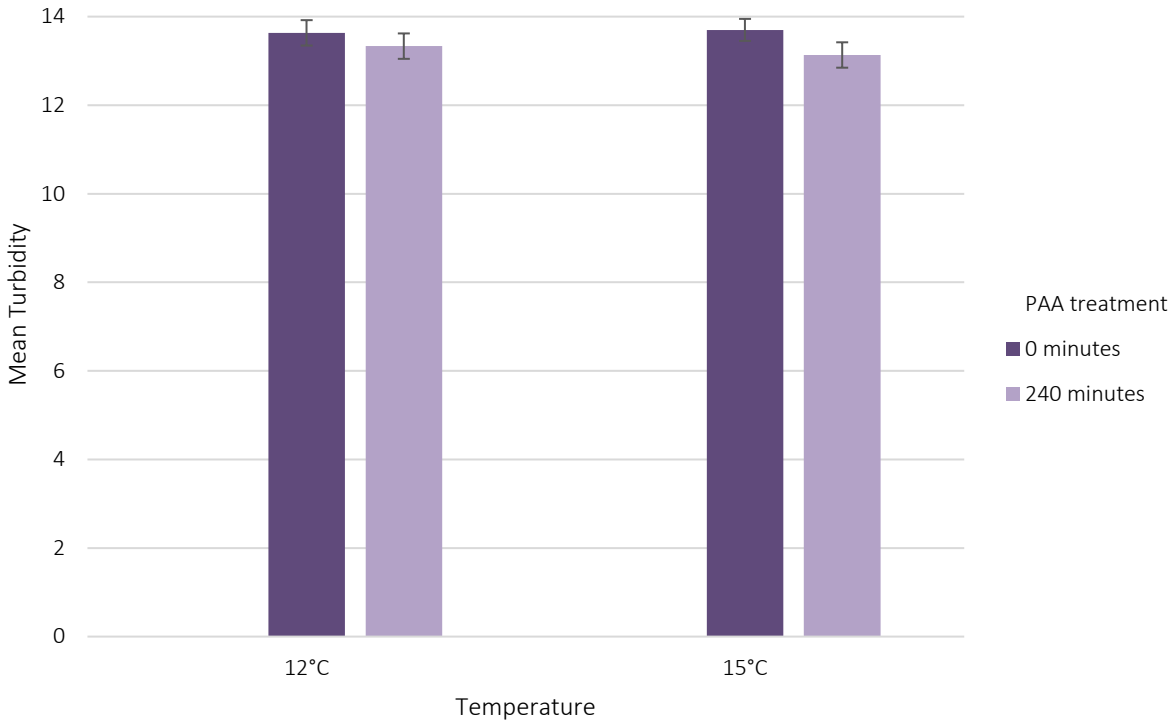

Figure 16. Averages of turbidity from triplicates of PAA-naive water samples at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C and 15°C

4.4.3 Water samples with different feed loads

At lower concentrations (10 mg/l, 15 mg/l, and 20 mg/l), the COD in the water samples with different feed loads decreased after 240 minutes of 2 mg/l PAA treatment. However, at higher concentrations (30 mg/l to 80 mg/l), the COD increased in a similar fashion as the RAS water samples.

Figure 17 and Figure 18 show the COD change after PAA treatment on the samples with different feed loads at 12°C and 15°C respectively. The results of a two-way ANOVA showed that the model is highly significant ($p < 0.001$). The intercept has a significant effect on the dependent variable, while temperature and PAA treatment had no significant effect. Feed loads in the samples had a significant effect on the dependent variable, meaning that the concentration of feed solution explains a significant proportion of the variation in the change in COD. The R - squared value of

.950 indicates that the model explains 95% of the variation, and the Adjusted R - squared value of .929 suggests a good fit for the model.

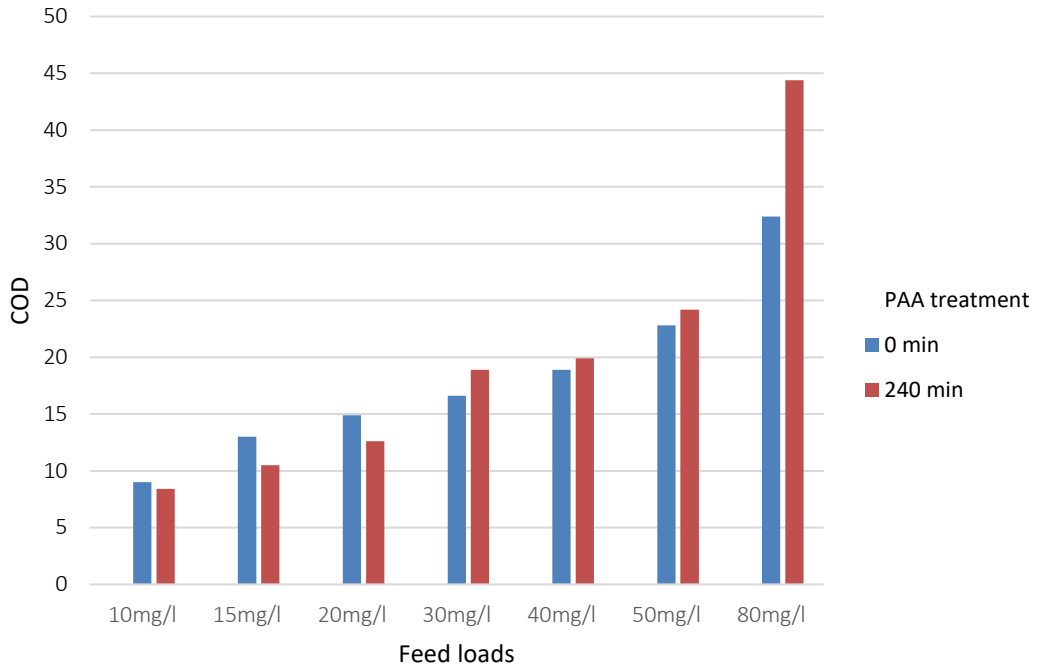

Figure 17. COD in the samples with 7 different feed loads at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C.

There was approximately 30 to 70 % reduction in TSS across the feed levels after 240 minutes of 2 mg/l PAA treatment at both temperatures. A two-way ANOVA was conducted to investigate the effects of Temperature, PAA treatment, and Feed loads on TSS. The TSS before and after PAA treatment for all feed loads are illustrated in Figure 19 and Figure 20 for treatment at 12°C and at 15°C respectively. The results of the analysis revealed that the model is significant ($p < 0.05$). The intercept is also significant ($p < 0.05$), indicating that it plays a crucial role in predicting TSS. The Partial Eta Squared values for Temperature and Feed loads were 0.006 and 0.428, respectively, suggesting that concentration of Feed loads had a stronger effect on TSS than temperature.

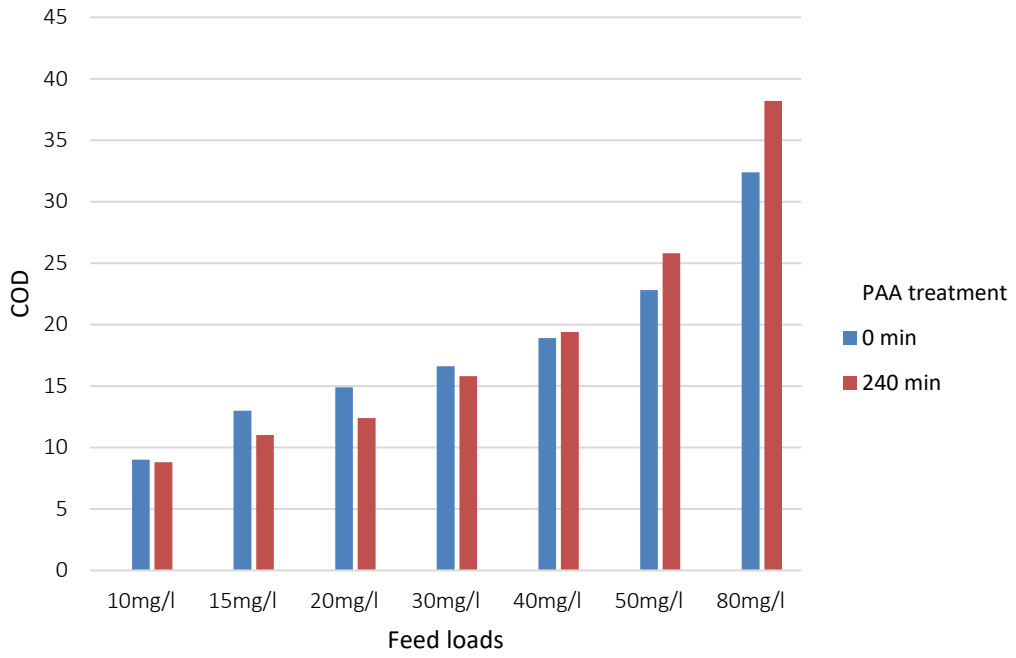

Figure 18. COD in the samples with 7 different feed loads at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 15°C.

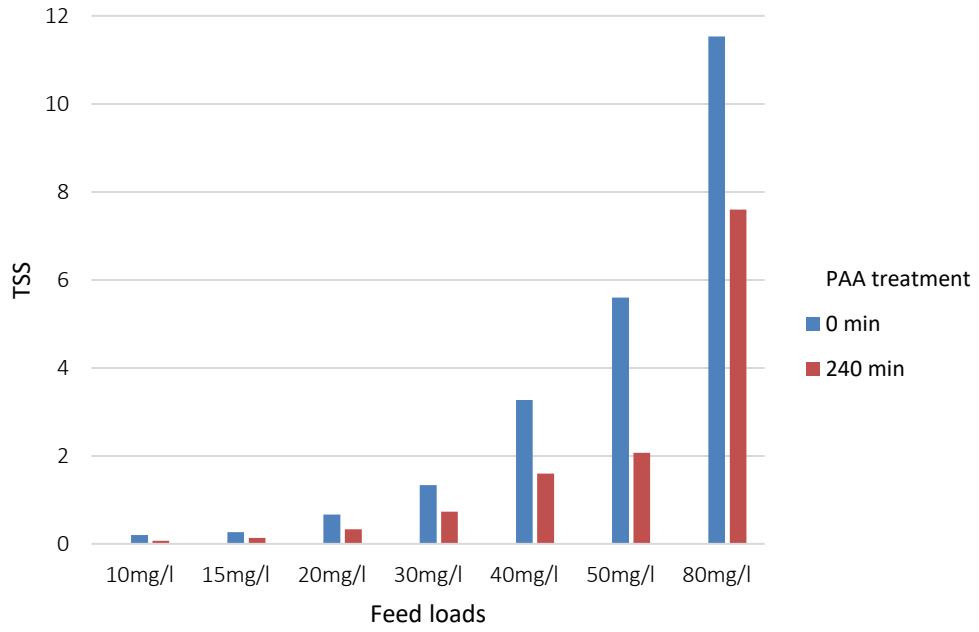

Figure 19. TSS in the samples with 7 different feed loads at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C

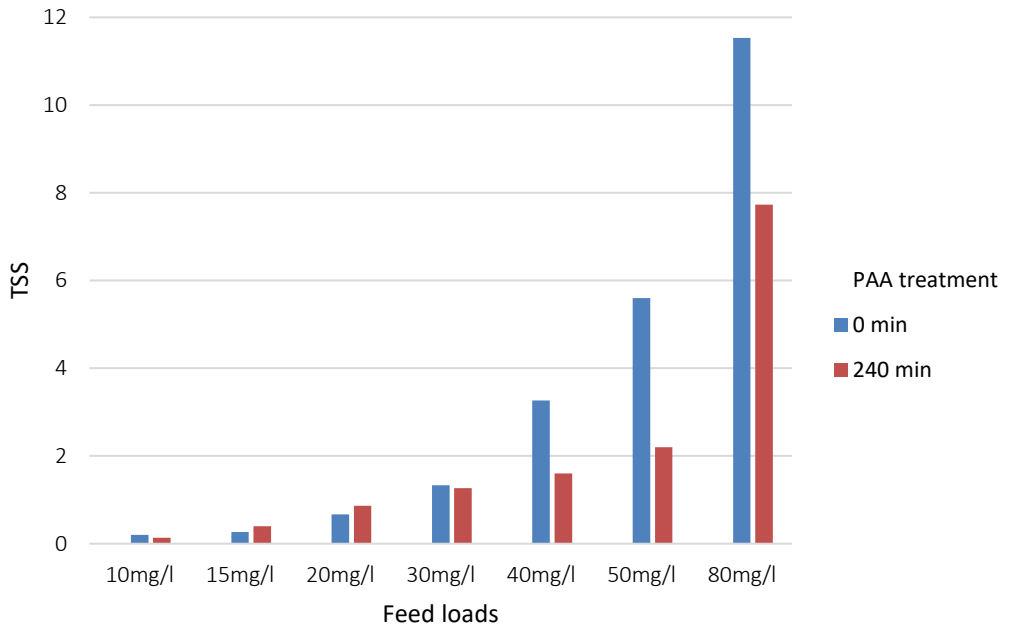

Figure 20. TSS in the samples with 7 different feed loads at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 15°C.

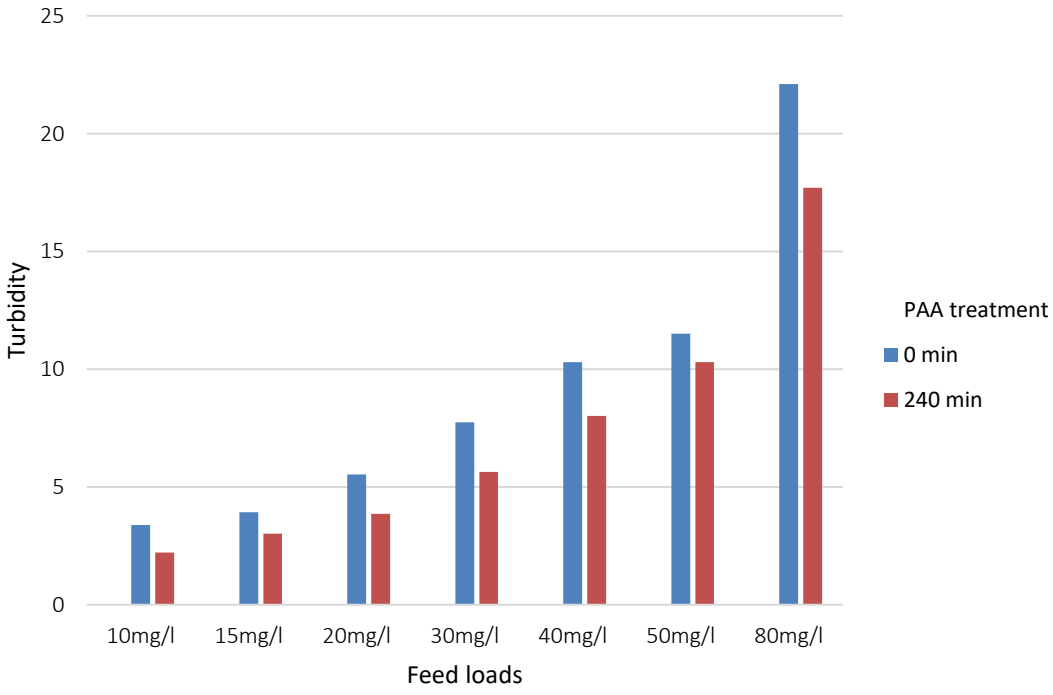

Figure 21. Turbidity in the samples with 7 different feed loads at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 12°C.

The turbidity decreased across all feed levels after 240 minutes of 2 mg/l PAA treatment, the turbidity levels of the samples with different feed loads are summarised in the Figure 21 and Figure 22 before and after PAA treatment at 12°C and at 15°C respectively. The results from a two-way ANOVA analysis on the turbidity values indicated that the model as a whole is statistically significant ($p < 0.001$), indicating that the independent variables collectively explain a significant amount of the variation in Turbidity. The results also showed that both Temperature and concentration of the Feed loads were significant predictors of Turbidity ($p < 0.001$). The "R Squared" value indicates the proportion of variance in the dependent variable that is accounted for by the independent variables in the model, which is 0.973, meaning that the independent variables explain 97.3% of the variance in Turbidity.

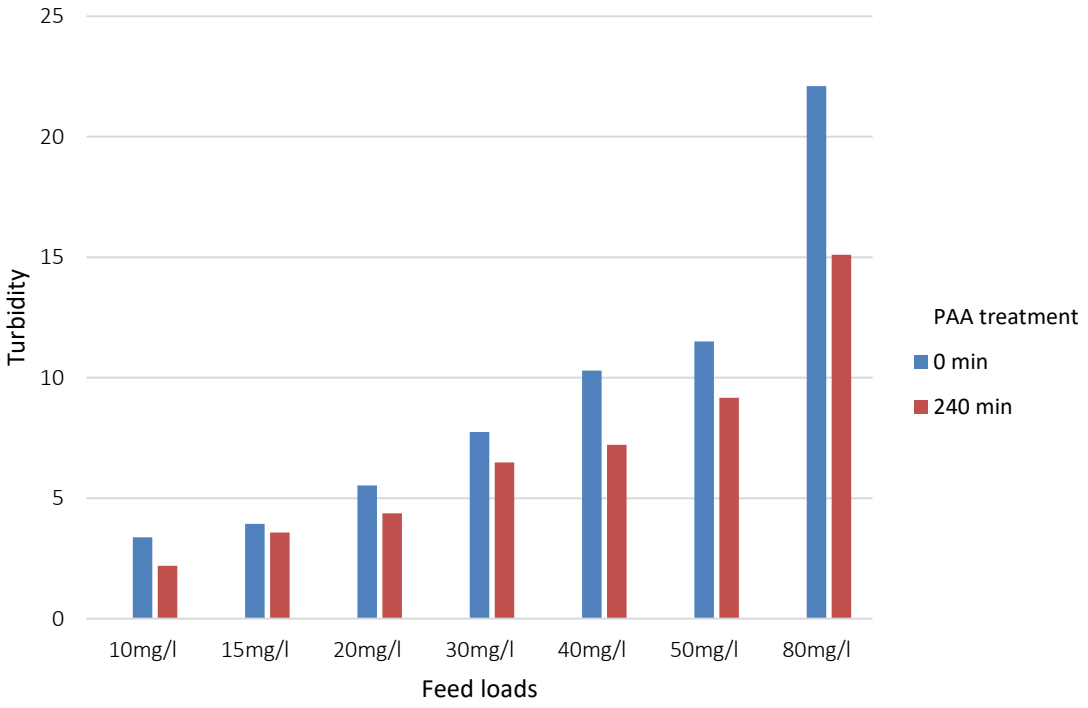

Figure 22. Turbidity in the samples with 7 different feed loads at time = 0 minutes and at time = 240 minutes after treatment with 2 mg/l PAA at 15°C.

5. Discussions

PAA is primarily degraded by chemical oxidation (Block, 1991) and its decay fits exponential first-order kinetics (Falsanisi et al., 2006). PAA degradation has been found to be dependent on the amount of organic matter in the system (Pedersen et al., 2009), with increasing levels of organic matter content resulting in increased PAA consumption and decreased microbial inactivation (Stampi et al., 2001). Aquaculture systems may employ the findings of disinfection efficiency and PAA consumption from municipal wastewater studies with secondary and tertiary effluents (Pedersen et al., 2009). Doses between 0.5 and 2 mg/l of PAA have been reported to have a high disinfection efficiency (Stampi et al., 2001).

Even though only sparse information is available on PAA decay in relation to temperature (Pedersen and Lazado, 2020), studies have indicated that temperature considerably affects the disintegration of PAA. Pedersen et al. (2013) found positive temperature-decay correlations for the decay of PAA. The rise in temperature has an increasing effect on the PAA decay kinetics (Amerian et al., 2019; Stampi et al., 2001). This might explain the rapid PAA consumption in the samples treated at 15°C than those treated at 12°C at the beginning of the PAA treatment.

5.1 Consumption of PAA in an adapted and a naive RAS water sample

PAA can potentially produce favourable conditions for some microbial populations. It is also known to increase the organic content in the water sample. (Kitis, 2004) This could justify the much more rapid decay of PAA in an adapted system than in a naive system. Given that most of the added PAA was consumed within the first 10 minutes of the treatment in the PAA - adapted sample, it might be much more difficult to maintain the residual levels of PAA in an adapted system long enough to achieve appropriate disinfection. With regards to this finding, a pulse application of PAA in freshwater RAS might be a wiser approach than a continuous application. Liu et al. (2017) reported that continuous application of PAA led to excess biofilm formation in flow-through tanks used for rainbow trout culture as opposed to a pulse application strategy.

Pulse application uses higher concentrations of PAA for shorter durations, resulting in minimal but adaptive stress in fish, partly inhibited nitrification, and minimal hyperplasia in the gills. The growth and immunity of the fish are unaffected. However, pulse PAA applications can induce oxidative stress, which can be minimized by periodic recoveries during application intervals. On the other hand, a continuous application is meant to maintain a concentration of 0.2 mg/l which can enhance biofilm formation, which may cause blockages and increase opportunistic pathogens. (Liu et al., 2017)

Rapid decay is advantageous in an environmental context, but it poses a challenge to the farmers and the aquaculture industry due to the large discrepancy between delivered quantities and realised residuals that it can cause (Pedersen et al., 2013).

5.2 Consumption of PAA in samples with different feed loads.

Previous studies have shown that PAA decay is subject to organic load which explains the consumption pattern of PAA in the samples with varying feed loads, with faster degradation at the beginning of the treatment at higher concentrations of feed. According to Pedersen et al. (2009), the organic matter content of the water and the presence of a biofilm on the surface are positively correlated with the disinfection demand of PAA (initial consumption and decay) under aquaculture conditions.

However, PAA decay in RAS is also subject to other parameters like temperature (Pedersen et al., 2013), light, fish stocking density (Pedersen et al., 2009), presence of transition metals, mode of addition, pH (Yuan et al., 1997) contact time, et cetera. RAS water samples have many variables that might influence the consumption of PAA than samples prepared with the addition of feed on DI water. While preparing the samples with different feed loads, it was attempted to match the water quality parameters like COD, turbidity and TSS of some feed levels to the RAS water samples. The PAA - adapted samples had similar TSS and COD as samples with 80 mg/l of feed load and turbidity similar to samples with 20 mg/l of feed load. Similarly, the PAA - naive samples had turbidity, TSS, and COD values close to the samples with a 50 mg/l feed load. However, the decay rate didn’t match even remotely between RAS water samples and feed samples with the

decay of PAA much faster and higher in RAS water samples. The findings suggest that feed as an organic content in RAS contributes to the consumption of added PAA, however, it is not the sole or the most important factor.

5.3 Changes in water quality parameters as a result of PAA treatment

When water treatment and hygiene procedures must be planned, it is crucial to consider the impacts of water quality to match anticipated and delivered active substances (Rach et al., 1997). It has been demonstrated by previous studies that the organic content in the water has a significant effect on PAA decay.

Both TSS and turbidity decreased after 240 minutes of PAA treatment across all the sample systems in this study. This was in alignment with the study done by Suurnäkki et al. (2020). The study by Suurnäkki et al. (2020), conducted with a Rainbow trout model, reported that the increased PAA application rate resulted in decreased turbidity and total suspended solids concentrations except in units with a PAA addition of twice per week, where values were closer to control units than other PAA application units. The decrease in turbidity might be explained by the higher potential of H_2O_2 to degrade organic matter which might have resulted in fewer larger particles in the water samples after PAA treatment (Yao et al., 2014). However, this finding contradicted with another study by Davidson et al. (2019) which reported that, regardless of the dosing interval, peracetic acid did not lower the total suspended solids (TSS) levels in the culture water. The TSS reductions with ozone in fish production systems contrast with this (Rueter and Johnson, 1995). It was speculated that periodic bacterial blooms of *Flectobacillus roseus* (non-pathogenic bacterium to Rainbow trout which was the model animal for the experiment), which is present in sufficient numbers to cause periodic increases in the visual turbidity of the culture water in both treatments, maybe the cause of the elevated TSS levels. (Davidson et al., 2019)

COD rises in proportion to the concentration of organic material (Jones, 2020). According to Kitis (2004), increased organic content in the effluent due to the acetic acid component and the

potential for microbial regrowth are drawbacks of PAA as a disinfectant in the wastewater industry. This can explain the surge in COD across the samples after PAA treatment.

6. Conclusion

In conclusion, the study examined the effects of peracetic acid (PAA) treatment on water quality parameters in recirculating aquaculture systems (RAS) at different temperatures. The results showed that PAA decayed more rapidly in PAA-adapted RAS water samples compared to PAA-naive samples, regardless of the temperature. Moreover, the decay of PAA was faster at the start at 15°C than at 12°C. The consumption of PAA was rapid at the beginning for the samples with higher feed loads. The PAA treatment resulted in an increase in COD and a significant decrease in TSS and turbidity. The effect of temperature on water quality parameters was significant, with higher temperatures resulting in more significant changes. It can be concluded that the results rejected all the null hypotheses and accepted the alternative hypotheses tested to support the thesis objectives. The findings presented here can provide valuable insights into the use of PAA for water treatment in RAS and can be useful in developing effective treatment strategies for sustainable aquaculture production.

7. Recommendations for further work

The decay of PAA in a sample with water as the delivery matrix is subject to multiple variables. It is observed from this study and from previous findings that it might not be possible to estimate accurate dosing by considering just one or two of those variables. PAA, however, is still a very potent disinfectant and further studies must be conducted in understanding it’s decay kinetics as fully as possible. Since, it might not be feasible to utilise PAA without completely understanding it's decay kinetics, how it behaves in different sample systems and its dose requirement; the following recommendations are suggested for further studies.

1. A comprehensive study must be carried out to quantify how and how much each variable (Microbial load, feed load, organic content of water, inorganic content of water,

temperature, pH, alkalinity, metabolic compounds, et cetera) in freshwater RAS affects the decay kinetics of PAA. The effect of maximum number of possible variables must be pooled to quantify and recommend appropriate dosing of PAA that will leave enough residuals to properly disinfect in the required contact time without harming the cultured organism.

2. Focus must be given to the pulse application of PAA in RAS and further studies are required to confirm the specifics and variables involved.
3. It might be useful to conduct a thorough study on which microflora flourishes as a result of PAA addition in RAS. The knowledge on selective microbial growth due to the PAA acting as a substrate could prove to be useful to not only understand the decay kinetics better but also to optimise all other treatment components of RAS simultaneously.
4. Numerous studies have already been performed to understand the effect of PAA in fish welfare and health in fish cultured in RAS and the concentration of PAA that can be utilised in RAS without harming the cultured organism has been determined. This must be taken into consideration while conducting new trials.

8. References

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Appendix

Appendix 1. Analytical procedure for COD cell test using Spectroquant Prove 100

COD
Chemical Oxygen Demand

114560
Cell Test

Measuring range:	4.0 – 40.0 mg/l COD or O_2 Expression of results also possible in mmol/l

Suspend the bottom sediment in the cell by swirling.

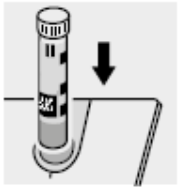
Carefully pipette 3.0 ml of the sample into a reaction cell, close tightly with the screw cap, and mix vigorously. **Caution, the cell becomes hot!**

Heat the reaction cell in the thermoreactor at 148 °C for 2 hours.

Remove the cell from the thermoreactor and place in a test-tube rack to cool.

Swirl the cell after 10 minutes.

Replace the cell in the rack for complete cooling to room temperature. **Very important!**



Place the cell into the cell compartment. Align the mark on the cell with that on the photometer.

Note:

To increase the accuracy is recommended to measure against an own prepared blank sample (reaction cell + COD-free water).

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) we recommended to use Spectroquant® CombiCheck 50, Cat.No. 114695, or the Standard solution for photometric applications, CRM, Cat.No. 125028.

To check for sample-dependent effects the use of addition solutions (e.g. in CombiCheck 50) is highly recommended.

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway