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Water Quality in RAS for Salmonids and Performance of MBBR

—*Case Study at Vik Settefisk AS*

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Master thesis (60 credits)

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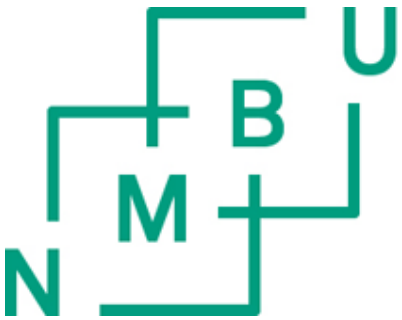

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Abstract

The purposes of this study was to find out water quality variation at Vik Settefisk AS, a land-based commercial smolts farm located in Bergen. In addition, the aim of the study was to evaluate nitrification efficiency in moving bed biofilm reactor (MBBR), disinfection efficiency of ozonation and UV irradiation, and to evaluate whether turbidity could produce a satisfactory estimate of total suspended solids.

There were four tests carried out during the study. Water samples were collected at different sites in the water treatment part. Measured parameters were temperature, pH, dissolved oxygen, alkalinity, NH_4-N, NO_2-N, NO_3-N, COD, turbidity, total suspended solids and heterotrophic bacteria count.

The results showed there were significant declines in TAN, free ammonia, COD concentration and turbidity in reused water after treatment ($P<0.05$). Suspended solids concentration in test 3 and 4 were lower than in test 1 and 2. High TAN concentration was observed in test 2 due to overfeeding, which was 16.32±0.17 mg/L at site 3.

MBBR functioned effectively in nitrogenous waste removal. COD/TAN ratio was low and stable in test 2 (ranged from 3.92±0.03 to 4.09±0.02). While in other tests, COD/TAN ratio surged from site 3 to 6, especially between site 5 and 6. The highest areal TAN removal rate (0.513±0.186 $g/m^2.d$) was observed in test 2.

In general, chamber 1 had higher efficiency in areal TAN, NO_2-N and COD removal rate than chamber 2. However when regarding percent TAN reduction, more TAN was removed in chamber 2 (41.62±1.81% to 59.58±3.71%) than in chamber 1(10.30±1.12 % to 30.53±7.45%), except in test 2. This was because chamber 2 had lager surface area than chamber 1 (58571 m^2 compared to 17677 m^2), and water had two-times longer retention time in chamber 2.

Make-up water had low heterotrophic bacteria count, which ranged from 4.7±2.5 to 60.0±35.6 CFU/mL before treatment. However, not even a 1-Log10 (90%) reduction was achieved in make-up water after ozone and UV treatment. In reused water, the result showed no significant decline in the heterotrophic bacteria count, the value ranged from 366.7±499.7 to 3633.3± 793.0 CFU/mL after ozonation.

There was strong positive correlation between TSS concentration and turbidity in a log-linear model (R^2 =0.917), with a regression equation of TSS = 15.46 ln (NTU) -8.4207. It suggested that turbidity could be used as a proxy for TSS in this study.

Key words: water quality variation, recirculating aquaculture system (RAS), MBBR, areal TAN removal rate, suspended solids, disinfection efficiency.

Abbreviations

ASL	Ammonium Surface Load
ATR	Areal TAN Removal
C/N	Carbon to Nitrogen ratio
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization of the United Nation
FCR	Feed Conversion Ratio
FLR	Feed Loading Rate
MBBR	Moving Bed Biofilm Reactor
NH_4-N	Ammonia Nitrogen
NO_2-N	Nitrite Nitrogen
NO_3-N	Nitrate Nitrogen
NTU	Nephelometric Turbidity Units
PC	Protein Concentration in feed
PE	Polyethylene
PP	Polypropylene
P_{TAN}	Production rate of Total Ammonia Nitrogen
RAS	Recirculating Aquaculture System
RBC	Rotating Biological Contactors
SGR	Specific Growth Rate
TAN	Total Ammonia Nitrogen
TSS	Total Suspended Solids
US-EPA	United State Environmental Protection Agency
UV	Ultra Violet

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1. INTRODUCTION

Aquaculture is the farming of aquatic organisms such as fish, crustaceans, molluscs and aquatic plants, the worldwide demand for fish has provided impetus to rapid growth in aquaculture (Timmons *et al.*, 2002). In 2012, there were 66.6 million tons of fish produced by aquaculture, it accounted for 42.2% of world food fish production. In addition, aquaculture is one of the fastest growing food-producing sectors, with averaged 6.5 % growth in the period from 2000 to 2012 (FAO, 2014).

Aquaculture systems can be classified into three main categories: extensive, semi-intensive and intensive, based on production per unit volume (m^3) or unit area (m^2) (Lekang, 2008). Natural small lakes fall in typical extensive systems, pond culture with feeding or aeration in semi-intensive, and recirculating aquaculture systems are in intensive.

Recirculating aquaculture systems (RAS) are tank-based systems in which environmental parameters are totally controlled, so fish can be stocked at high density. RAS technology has been developed and refined for the last three decades (Molleda *et al.*, 2007). RAS technology has capability to work at high capacity with less water and area requirement as compared with traditional fish farming, also RAS can reduces chemical and antibiotic usage and waste disposal; in addition, RAS is species-adaptable, this means fish can be produced year-round (Helfrich and Libey,1991; Masser *et al.*, 1999; Timmons *et al.*, 2002) . However, RAS needs high capital and operational investment that is the main demerit. Moreover, it is a complex system for startup and expertise is needed to maintain and monitor. (Masser *et al*., 1999).

Water quality control in RAS achieved by many different components. In general, RAS consists of heater or heat exchanger to adjust water temperature, aeration system to reduce dissolved CO_2 concentration, oxygenation system to supply sufficient oxygen, drum filters to remove suspended solids, disinfection system (UV and ozone equipment) to inactivate pathogens and bio filter system to remove nitrogen waste. Alkalinity in the system is controlled by adding chemicals into it (Ding, 2012).

By FAO report (2014), it has been observed that farming of salmon and rainbow trout has developed into a major business in the Norwegian coast. Norway produces nearly 1 million tons of salmon annually, and the industry aims to produce 2.5 million tons salmon within the next decade (Drengstig, 2011).

In 2009, more than 230 million salmon smolts and 15 million trout smolts were produced, which has increase by 50 million since 2006. However, less than 10% of these smolts were produced in recirculating aquaculture system, the majority are being produced in flow-through systems (Drengstig, 2011). This means RAS can be a promising trend of smolts production in Norway. Figure 1.1 shows the annual production of salmon and rainbow trout smolts in Norway in the period from 1999 to 2009.

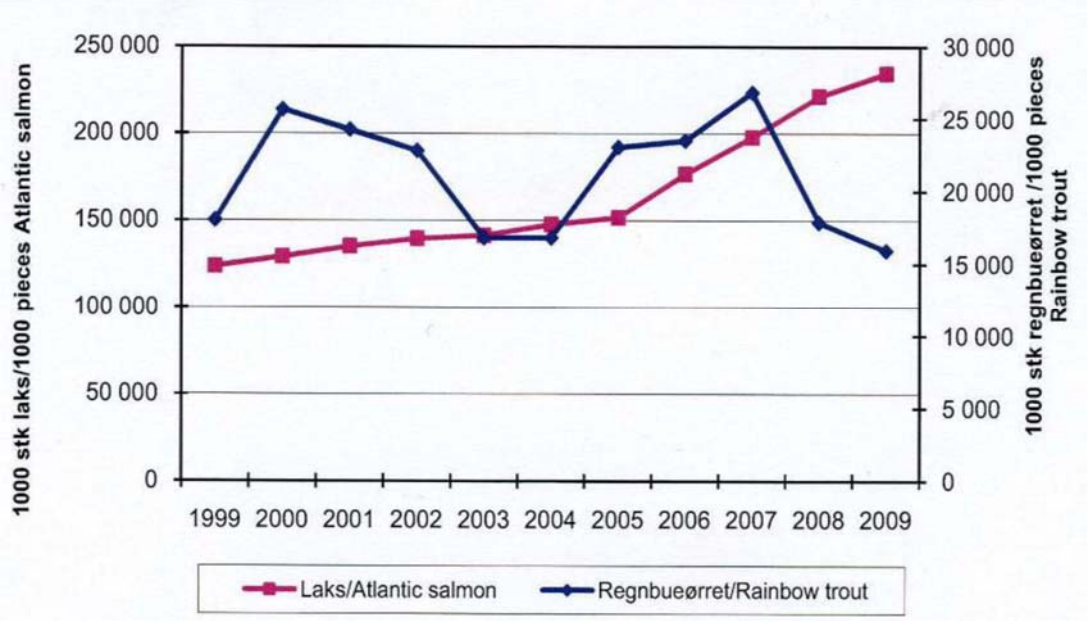

Figure 1.1 Annual production of salmon and rainbow trout smolts in Norway during 1999-2009 (Drengstig, 2011).

1.1 Objective

- To determine water quality variation in a commercial smolts farm employing recirculating aquaculture system and how is water quality being reconditioned in order to be reused,
- To study the nitrification efficiency in moving bed biofilm reactor (MBBR) and changes in suspended solids and turbidity during the treatment,
- To study disinfection efficiency of ozonation and UV irradiation on make-up water, and disinfection efficiency of ozonation on reused water,
- To evaluate whether turbidity could produce a satisfactory estimate of total suspended solids at Vik Settefisk AS.

2. LITERATURE REVIEW

2.1 Water quality in RAS and water quality requirement for salmonids

Optimal and stable water quality is one of the most important factors to successful aquaculture. One of the major advantages of RAS is the ability to control environment factors and optimize water quality (Timmons *et al.*, 2002). The critical and decisive parameters of water quality in aquaculture are: temperature, pH, alkalinity, dissolved oxygen, carbon dioxide, ammonia, nitrite and suspended solids (Colt, 2006).

Depending on farmed species, life stage and farming conditions, different water quality criteria will be used (Colt, 2006). Table 2.1 shows the recommended water quality requirement of recirculating aquaculture system (Masser *et al.*, 1999).

For salmonids, based on gill damage caused by ammonia exposure, the recommended un-ionized ammonia criterion in salmonid culture is only 0.0125 mg/L (Westers, 1981). The optimal temperature for rainbow trout is 14-16℃, while for Atlantic salmon is 15℃ (Aston *et al.*, 1982). Fivelstad *et al.* (2003) found increased incidences of nephrocalcinosis when salmon were exposed 16 and 24 mg/L carbon dioxide after 58 days.

Table 2.1 Recommended water quality requirement of recirculating aquaculture system (Masser *et al.*, 1999).

Parameters	Recommended value or range
Temperature	Optimum range for species cultured–less than 5℉ 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%

2.2 Description of Moving Bed Biofilm Reactor (MBBR)

There are many types of biofilm systems used for water treatment, such as trickling biofilters, rotating biological contactors (RBC), granular media biofilters, floating bead biofilters and fluidized bed biofilters (Timmons *et al.*, 2002), they all have advantages and disadvantages. The trickling filter is not volume-effective; mechanical failures have often been experienced in rotating biological contactors; granular media biofilters need periodic back flashing and the fluidized bed reactors show hydraulic instability (Rusten *et al.*, 2006). In this context, the moving bed biofilm reactor (MBBR) technology was developed in the late 1980s and early 1990s in Norway (Ødgaard *et al.,* 1999).

Now MBBR has been applied world-widely for treatment of municipal and industrial wastewaters, as well as for water treatment in aquaculture (Rusten *et al.*, 2006). In aquaculture industry, MBBR is mainly applied for nitrification, as well as removal of organic matters. In order to avoid the heterotrophic bacteria that consume organic matters suppressing the nitrifying bacteria at high organic loads, MBBR is always operated at low organic loads in aquaculture system (Rusten *et al.*, 2006).

Compared with most other biofilm reactors, MBBR utilizes the whole tank volume for biomass growth, it also has an insignificant head-loss and no need for periodic backwashing and not susceptible for clogging (Rusten *et al.*, 2006). In addition, the filling fraction of biofilm carriers in the reactor can be subject to preferences. However, it is recommended that filling fractions should be less than 70 % to keep the carrier suspended freely in reactor (Ding, 2012).

MBBR is a technology based on biofilm theory, with an active biofilm growing on specially designed plastic carriers (or biomedia) that are suspended in the reactor. It can be operated both in aerobic and anaerobic conditions, as illustrated in Figure 2.2.1. In aerobic case, the biomedia are kept suspended by agitation from aeration diffusers, while in anaerobic case, a mixer is used to keep the biomedia moving (Ødgaard *et al.,* 1999). Bio-medias are made from different materials and high-density polyethylene is commonly used, which has a density about

0.95g/cm^3. In order to provide maximum specific surface area (m^2/m^3), bio-medias are designed in various shapes and sizes (Ding, 2012). Figure 2.2.2 shows a commonly used biomedia K1 with specific surface area 350 m^2/m^3.

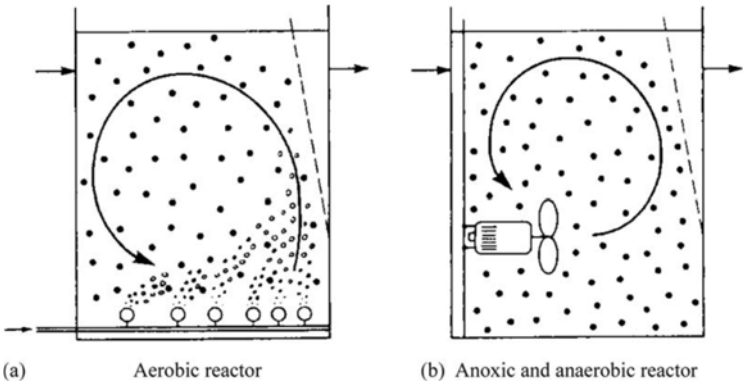

Figure 2.2.1 Illustration of the moving bed biofilm reactors (Ødgaard *et al.,* 1999)

Figure 2.2.2 Biomedia K1

The nitrification process in MBBR is influenced by many environmental factors, such as temperature, pH, alkalinity, COD level, the dissolved oxygen (DO) level in the reactor, the total ammonium nitrogen (TAN) level, and the status of the biofilm(Rusten *et al.*, 2006). To evaluate the efficiency of TAN removal in reactor, there are three parameters commomly used (Pfeiffer and Wills, 2011): 1) volume TAN removal rate (g TAN m^{-3}d^{-1}), 2) areal TAN removal rate (g TAN m^{-2}d^{-1}), 3) and percent TAN removal efficiency.

2.3 Nitrification process

2.3.1 NH_3 and NH_4^+ equilibrium in water

One of the major end product of protein metabolism in fish is ammonia, it is mainly excreted by the gill of fish as un-ionized ammonia (or free ammonia, NH_3), and a small amount is excreted in urea as ionized ammonium (NH_4^+). Therefore, ammonia exists in two forms in water: un-ionized ammonia (NH_3) and ionized ammonium (NH_4^+). The percentage of each form is dependent on pH, temperature and salinity (Anthonisen *et al.*, 1976). As showed in Table 2.3.1(US-EPA, 1985), when pH increases by one unit (e.g., from 6 to 7) at 15 °C, the percentage of free ammonia increase more than 10 times.

Table 2.3.1. Percent (%) NH_3 in aqueous ammonia solutions at varying pH and water temperature (°C) (US-EPA, 1985)

	Temperature											
pH	13	14	15	16	17	18	19	20	21	22	23	24
6.0	0.024	0.025	0.027	0.030	0.032	0.034	0.037	0.040	0.043	0.046	0.049	0.053
6.5	0.074	0.080	0.086	0.093	0.101	0.108	0.117	0.125	0.135	0.145	0.156	0.167
7.0	0.235	0.253	0.273	0.294	0.317	0.342	0.368	0.396	0.425	0.457	0.491	0.527
7.5	0.738	0.796	0.859	0.925	0.996	1.07	1.15	1.24	1.33	1.43	1.54	1.65
8.0	2.30	2.48	2.67	2.87	3.08	3.31	3.56	3.82	4.10	4.39	4.70	5.03

The sum of this two forms is called total ammonium nitrogen, or simply TAN (TAN = NH_4–N + NH_3–N). Both NH_3 and NH_4^+ are toxic to fish, however unionized ammonia is more concerned in aquaculture (Körner *et al.*, 2001). The toxicity concentration of free ammonia to fish depends on the fish species, life stage, size, concentration of fine solids, refractory organics, surface-active compounds, heavy metals, and nitrate level in water (Colt, 2006). In most cases, the acceptable level of unionized ammonia in recirculating aquaculture systems is only 0.025 mg /L (Neori *et al.*, 2004; Chen *et al.*, 2006).

Total ammonia nitrogen generated per day in an aquaculture production system can be calculated based upon the feeding rate (Timmons *et al.*, 2002), as shown in the following equation:

$$P_{TAN} = F \times PC \times 0.092$$

Where:

P_{TAN} Production rate of total ammonia nitrogen (kg/day)

F Feed rate (kg/day)

PC Protein concentration in feed (decimal value)

The constant 0.092 in the equation assumes that there are 16% N in the feed protein, 80% N is assimilated by fish, 80% assimilated N is excreted, and 90% of N excreted as TAN+10% as urea. In addition, feces and uneaten feed in tanks are removed quickly by sedimentation or filtration.

2.3.2 Nitrification process description

Nitrification is an important process in the cycling of nitrogen. There are three nitrogen conversion pathways that normally existed in aquaculture systems for the removal of ammonia–nitrogen. They are:

*Photoautotrophic removal by algae;

*Autotrophic bacterial conversion of ammonia–nitrogen to nitrate–nitrogen;

*Heterotrophic bacterial conversion of ammonia–nitrogen to microbial biomass.

The nitrification process is carried out by nitrifying bacteria and it has been well studied, nitrifying bacteria are chemoautotrophic and they get energy for life process from nitrification reaction (Barnes and Bliss, 1983; Wiesmann, 1994).

First free ammonia is oxidized to nitrite by ammonia oxidizing bacteria genera (such as Nitrosomonas, Nitrosospira, and Nitrosococcus), as shown in Equation 2.1. Then nitrite is oxidized to less toxic nitrate by nitrite oxidizing bacteria genera (such as Nitrobacter and Nitrospira), as showed in Equation 2.2. These reactions will consume oxygen and produce hydrogen ions (which would result in decline of pH).

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$…….....….Equation 2.1

$NO_2^- + 0.5O_2 \rightarrow NO_3^-$…….Equation 2.2

According to US-EPA (1984), the complete nitrification process can be express as:

$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N + 0.98\ NO_3^- + 1.041\ H_2O + 1.88\ HCO_3^-$

…….Equation 2.3

Here $C_5H_7O_2N$ presents the chemical composition of nitrifying bacteria. From Equation 2.3, we know that for every gram of TAN being oxidized to nitrate nitrogen, approximately 4.18 g of oxygen and 7.07 g of alkalinity (as $CaCO_3$) are consumed and 0.17 g nitrifying bacteria biomass are produced (Chen *et al.*, 2006).

Heterotrophic bacterial also present in water, their growth will be stimulated at high concentration of organic substrate. At high carbon to nitrogen(C/N) feed ratio, heterotrophic bacteria can also assimilate ammonia-nitrogen directly into cellular protein (Ebeling *et al.*, 2006). Lipponen *et al.* (2004) and Summerfelt *et al.* (2004) reported that heterotrophic bacteria could assimilate the ammonia and participate in the process of biofilm building, by utilizing soluble organic carbon.

2.3.3 Effect of alkalinity on nitrification rate

As shown in Equation 2.3, HCO_3^- is being consumed in nitrification process constantly. For every kilogram of feed consumed by fish, approximately 0.15–0.19 kg sodium bicarbonate ($NaHCO_3$) needs to be added into water (Davidson *et al.*, 2011). If the alkalinity loss is not compensated by supplementation with a base (such as sodium hydroxide or sodium bicarbonate), the alkalinity and pH of the system will decrease gradually (Loyless and Malone, 1997).

In addition, Paz (2000) and Biesterfeld *et al.* (2003) found that maintaining adequate alkalinity concentrations is critical for sustainable nitrification. In a bench-scale experiment performed in a turbot farm using moving bed biological reactor(MBBR), Rusten *et al.* (2006) found that the nitrification rate dropped to only half of the original rate when alkalinity dropped from approximately 115 mg/L as $CaCO_3$(pH=7.3) to 57 mg/L (pH=6.7). Villaverde *et al.* (1997) reported a linear increase in nitrification efficiency of 13% per unit pH increase from pH 5.0 to 8.5.

Mydland *et al.* (2010) reported that if recirculating aquaculture system was operated with sub optimal alkalinity, theoretically it could encounter larger pH fluctuation, higher concentrations of TAN and NO_2–N due to accumulation, and microbial community instability, which is harmful to the fish. Especially for Atlantic salmon, which is sensitive to elevated concentrations of nitrite nitrogen without concurrent chloride adjustments (Gutierrez *et al.*, 2011).

2.3.4 Effect of C/N ratio on nitrification rate

At a high C/N ratios, the heterotrophic bacteria out-compete nitrifying bacteria (autotrophic) for available oxygen and space in the biofilters (Michaud *et al.*, 2006). One of the critical factors affecting the design and operation of a nitrification system is the ratio of the biodegradable organic carbon to the nitrogen, or C/N ratio (US-EPA, 1993). As previously mentioned in Section 2.3.2, there are three pathways in nitrogen cycle and two genres of bacteria are involved in nitrification. Autotrophic bacteria derive their energy from inorganic compounds and heterotrophic bacteria that derive energy from organic compounds (Hagopian and Riley, 1998). Actually, heterotrophic bacteria have a maximum growth rate significantly higher than nitrifying bacteria (US-EPA, 1993). Therefore, nitrification prefer a low C/N ratio.

2.3.5 Effect of PH on nitrification rate

Many authors have reported that the optimum pH range for nitrification is from 7.0 to 8.0 (Jones and Paskins, 1982; Painter and Loveless, 1983; Antoniou *et al.*, 1990). As showed in Table 2.3.2, the optimum pH range for Nitrosomonas is 7.9 - 8.2, and 7.2 – 7.6 for Nitrobacter (Alleman, 1984).

pH influences nitrifying bacteria in three ways. First is the activation - deactivation of nitrifying bacteria. The change of pH will lead to binding of H^+ or OH^- ions with the weak basic-acid groups and then blocking the active sites of nitrifying bacteria on biofilms (Quinlan, 1984).

Second is the influence on availability of mineral carbon nutritional, which is the carbon source for nitrifying autotrophic bacteria. Availability of carbon source is also related to alkalinity. However, pH plays an important role in carbon equilibrium.

The third effect is inhibition of free ammonia and free nitrous acid (Anthonisen *et al.*, 1976; Ford *et al.*, 1980), and heavy metals (Braam and Klapwijk, 1981; Nelson *et al.*, 1981). Concentrations of free ammonia and nitrous acid depends on temperature, pH, and the

concentrations ammonium and nitrite. Free ammonia concentration increases at high pH, whereas nitrous acid concentrations rises at low pH (Ford *et al.*, 1980).

2.3.6 Effect of Temperature on nitrification rate

As shown in Table 2.3.2, temperature has different effects on the growth rate of ammonium and nitrite oxidizers. When temperature is above 15°C, ammonium oxidizers grow faster than nitrite oxidizers. Only at temperatures above 25 °C is it possible that the ammonium oxidizers out-compete the nitrite oxidizers (Dongen *et al.*, 2001; Brouwer *et al.*, 1996).

Hellinga *et al.* (1998) reported that nitrite oxidizers can be selectively washed out in a system with low hydraulic retention time when the temperature is above 26 °C, while the ammonium oxidizers can grow fast enough to stay in the reactor. As previously mentioned, the pH has a strong influence on the system because in the low pH range the nitrite oxidizers grow faster than the ammonium oxidizers.

Table 2.3.2 Effect of the pH, temperature on nitrification process (Paredes *et al.*, 2007).

Factor	Effect
Temperature	
T＞15℃	Ammonium oxidizers grow faster than nitrite oxidizers.
T＞25℃	Ammonium oxidizers can out-compete nitrite oxidizer.
pH	
7.0–8.0	Optimum range for nitrification.
7.9–8.2	Optimum range for ammonium oxidizers (Nitrosomas).
7.2–7.6	Optimum range for nitrite oxidizers (Nitrobacter).

2.3.7 Effect of dissolved oxygen (DO) on nitrification rate

From the Equation 2.1 it can be seen that 2 mole of oxygen are needed for the complete oxidation of 1 mole of ammonia to nitrate (Canziani *et al.*, 2006). DO concentration is an important factor affecting nitrification (Stenstrom and Poduska, 1980).Continuous nitrification

under low DO will leads to nitrite accumulation, because nitrite oxidizers is more sensitive to oxygen than ammonia oxidizers (Jayamohan *et al.*, 1988).

Dissolved oxygen concentration is also an important factor and it is related to the thickness of the biofilm and temperature (Hao[a] *et al.*, 2002). With a defined ammonium surface load (ASL) under lower temperature, a thicker biofilm is required and, hence, a higher dissolved oxygen concentration is necessary in the reactor. A thin biofilm needs a lower dissolved oxygen concentration. Higher dissolved oxygen concentrations will cause total nitrification and a lower nitrogen removal rate (Koch *et al.*, 2000; Hao[b] *et al.*, 2002).

2.4 Disinfection by ozonation and UV irradiation

Ozone is a powerful oxidant which has been widely applied in RAS, especially within recently constructed intensive salmonid production systems (Summerfelt *et al.*, 2001). Ozone is added into aquaculture system waters for both disinfection and water quality improvement purposes (Wedemeyer, 1996). It works well in fish pathogens inactivation, organic wastes removal (including color and smell removal) and nitrite oxidization. Besides, ozonation of water in recirculating systems improves fish welfare by reducing fish disease and environmental sources of stress (Brazil, 1996).

At 20 ℃, the half-life of ozone dissolved in pure water is 165 min (Rice *et al.*, 1981). In recirculating aquaculture systems, where reused water contains high levels of organic material and nitrogen waste, will leads to an even shorter half-life time (e.g., <15 s), which makes maintaining a specific concentration of ozone residual difficult (Bullock *et al.*, 1997), therefore it has to be produced and used on site.

Ozone is generally produced by leading enriched oxygen feed gas through a high-voltage electrical corona. Pure oxygen is mostly being used because it is not only 2-3 times more energy-efficient when compared with using air (Masschelein, 1998), but also pure oxygen gas is often already used to maximize carrying capacity in most intensive fish farms. All typical oxygen transferring devices can be used to transfer ozone gas to water as well (Summerfelt and Hochheimer, 1997). Continuous liquid-phase transfer units are usually selected when the ozone residual must be kept for a certain time (Bellamy *et al.*, 1991). High column bubble diffusers are frequently used in fish farms and in this way more than 85% of ozone are transfer to the liquid phase (Liltved, 2001).

Ozonation can kill bacteria, virus and other microorganisms in water, but to get an ideal disinfection effect it requires keeping a certain dissolved ozone level for a given contact time(c*t effect). Literature reviews on ozone dosing requirements indicates that many pathogenic organism can be inactivated by an ozone c*t dosages of 0.5-5.0 min mg/L (Liltved, 2001). However, certain kinds of spore forming organism are difficult to inactivate by ozone. For this reason, to disinfect water in recirculating aquaculture systems thoroughly, it needs much greater ozone dosages than it is typically required for simply water quality control (Bullock *et al.*, 1997). Ozone can also been used to disinfect effluent from hatcheries or farms in order to prevent the potential release of fish pathogens to the receiving watershed (Liltved, 2001).

Although ozone has a rapid reaction rate and little harmful by-products, it is lethal to fish at a very low levels which may be as low as 0.01 mg/L, the maximum safe level of chronic ozone exposure for salmonids is 0.002 mg/L (Wedemeyer *et al.*, 1979). Compilation of results from several other studies shows that most fish exposed to ozone levels that more than 0.008-0.06 mg/L will develop severe gill damages which can result in serum osmolality imbalances or kill fish immediately or leave them more susceptible to pathogens (Bullock *et al.*, 1997). To avoid this problem, ozone residual can be removed by increasing the contact times, aeration and degassing, reaction with hydrogen peroxide, or intense UV light irradiation.

UV irradiation is also widely used in aquaculture industry to inactivate microorganisms (Sharrer *et al.*, 2005). Compared with ozone, using of UV light will not produce toxic residuals or form harmful byproducts to fish at all. UV light functions by breaking down the nucleic acids of microorganisms, which will result in death or function lose. Microorganism 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 wavelength from 250-260nm. According to Hunter *et al.* (1998), completely ozone residuals 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.

Most fish pathogens can be inactivated by UV doses of 30 mW s/cm^2 at 254nm. But according to required removal rate and targeted pathogens, the UV doses requirement ranges wildly from 2 mW s/cm^2 to 230 mW s/cm^2 (Wedemeyer, 1996). Actually, the real UV dose requirement depends largely on UV intensity, exposure time, water flow and transmittance of UV in water.

In order to get better disinfection, exposure time or UV intensity are often increased in practice, because UV transmittance is conversely reduced with increase in total suspended solids concentration (Loge *et al.*, 1996) and pathogens may be shield by envelop with particulate matter (Emerick *et al.*, 1999). Sharrer *et al.* (2005) presented a hypothesis that in reused aquaculture system where reused water is treated with UV irradiation may provide selection pressure for some bacteria species that merged together with particulate matter, because this provides protection from the UV irradiation.

Ozonation followed by UV irradiation has been applied in wastewater and drinking water treatment to get best removal of microorganisms for decades (White, 2005). In RAS, if certain amount of ozone is used to disinfection, it can prevent accumulation of fine particles in the system, which could subsequently improve the disinfection efficiency of UV irradiation. Research done by Sharrer and Summerfelt (2007) also indicated ozonation followed by UV irradiation provides effective bacteria inactivation in a freshwater recirculating system, combining ozone dosages of only 0.1–0.2 min mg/L with a UV irradiation dosage of approximately 50 mJ/cm^2 would consistently reduce bacteria counts to near zero.

To sum up, according to water quality and disinfection goal, attention must be paid when UV and ozone are used in fish farm, both the amount and contact time. UV plants are cheaper and less complex compared with ozone plant. In addition, there is no toxic byproduct or residual problems related with UV irradiation. However, when water is turbid, UV has little disinfection effect. In this case, ozone will still works well in oxidizing organic particle, removal of color and smell, as well as disinfection if ozone is abundant in amount. Therefore, ozonation and UV irradiation are always being used together in water treatment in RAS.

2.5 Oxygenation and carbon dioxide control in RAS

Pure oxygen has been used in aquaculture to intensify fish production since the 1970s (Speece, 1981). Oxygenation applied in intensive fish farming systems can increase the carrying capacity notably at a given water flow by removing oxygen concentration as the first limiting factor (Summerfelt *et al.*, 2000). The use of pure oxygen gas can also reduce production costs, by increasing carrying capacity and reducing water consumption.

Since pure oxygen is not inexpensive, oxygenation should be done at a proper way with high oxygen transfer efficiency and oxygen absorption efficiency. In general, oxygenation technology has been well developed and there are various equipment that suitable for different production system, for example, U-tubes, oxygenation cones and multi-staged low head oxygenators are widely used in recirculating aquaculture system. Oxygen supersaturated water should be injected to the bottom of fish tanks and be distributed evenly as soon as possible in the tank (Masser *et al.*, 1999).

For every mole oxygen being consumed by fish and bacteria in system, one mole carbon dioxide is produced. Furthermore, RAS has a relative low water exchange rates (1%-10%), and systems with oxygenation typically do not allow for the removal of carbon dioxide in large amount (Grace and Piedrahita, 1994). Therefore, in intensive recirculating aquaculture system where large amounts of pure oxygen are added into water, carbon dioxide accumulation is a practical problem (Summerfelt *et al.*, 2003).

High level of dissolved carbon dioxide is toxic to fish, elevated CO_2 level may decrease the ability of hemoglobin to transport oxygen (the Bohr effect), even higher level will decrease the maximum oxygen binding capacity of blood (the Root effect), and increase blood acidity (Jobling, 1994). Tolerance to dissolved carbon dioxide depends on fish species, life stage of the fish, and many other environmental factors, such as alkalinity, pH, and dissolved oxygen levels (Summerfelt *et al.*, 2000). Salmonids will be affected when dissolved carbon dioxide is approximately 20 mg/L, while tilapia and catfish will tolerate dissolved carbon dioxide levels up to 60 mg/L (Wedemeyer, 1996).

Since carbon dioxide is much more soluble than oxygen in water, it is essential that CO_2 stripping should be done before oxygenation. In practice, packed column aerators with forced ventilation are widely used, because they are more effective than diffuser aeration and sub-surface aerators (Colt and Orwicz, 1991). Packed column aerators are filled with packing (e.g., plastic balls) that can increase water-air contact surface and contact time. For most effective carbon dioxide stripping, at least 5-10 vol. air per vol. water should be contacted (Summerfelt *et al.*, 2000), this can be achieved by installing blower at the bottom of packed column aerator.

2.6 Effects of total suspended solids (TSS) and turbidity on salmonids

The term total suspended solids (TSS) refers to the mass (mg) or concentration (mg/L) of inorganic and organic matter which is held in the water by turbulence (Bilotta and Brazier, 2008). They are typically consisted of fine particles with a diameter less than 62 μm (Waters, 1995), and are measured directly by collection of sample water followed by filtration of this sample through a dried and pre-weighed 0.7 μm pore-size glass fiber-filter (Gray *et al.*, 2000) .

Suspended solids can cause water quality deterioration in many ways. Physically, TSS can result in reduced penetration of light and temperature changes (Ryan, 1991); Chemically, contaminants may be released due to TSS presence, such as heavy metals and pesticides (Dawson and Macklin, 1998); furthermore, if TSS have a high organic content, dissolved

oxygen will be consumed by in-situ decomposition, which may lead to low dissolved oxygen concentration and even kills fish (Ryan, 1991).

TSS can also affect the free-living fish directly, by clogging and being abrasive to fish gills (Cordone and Kelley, 1961), or stressing the fish and destroying their immune system which will result in increased disease susceptibility and osmotic dysfunction (Redding *et al.*, 1987). Migration of wild Salmonids can be influenced by TSS presence (Bisson and Bilby, 1982). Bilotta and Brazier (2008) compiled many research results and summarized the effects of various concentration, and durations of exposure to, suspended solids on salmonids, as shown in Table 2.6.1.

The effect of TSS on fish depends on four main factors, such as the concentration of TSS; the duration of exposure to TSS; the chemical composition of TSS and the particle-size distribution of TSS (Bilotta and Brazier, 2008). The real effects on salmonids will also differ based on life stage of salmon (Bash *et al.*, 2001).

Table 2.6.1 Summary of effects of various concentration, and durations of exposure to, suspended solids on salmonids (Bilotta and Brazier, 2008).

Species	SS level, mg/L	Duration of exposure, h	Effects on fish
Atlantic salmon	20	-	Increased foraging activity
Arctic grayling	25	24	6% mortality of sac fry
Rainbow trout	47	1,152	100% mortality of incubating eggs
Arctic grayling	65	24	15% mortality of sac fry
Atlantic salmon	60-180	-	Avoidance behavior
Arctic grayling	185	72	41% mortality of sac fry
Chinook salmon	488	96	50% mortality of smolts
Coho salmon	800-47,000	-	80% reduction in fertilization success
Coho salmon	2,000-3,000	192	Reduced feeding efficiency
Rainbow trout	pulses	456	Reduced growth
Brown trout	5,838	8,670	85% reduction in population size
Coho salmon	40,000	96	Physical damages to gills
Chinook salmon	207,000	1	100% mortality of juveniles

Turbidity is a measurement of light scattering properties of water. Due to low cost and ease of use, Nephelometric turbidity meters have been most widely applied in field study, and turbidity data are recorded in nephelometric turbidity units (NTU) (Lewis, 1996).

There are differences and correlations between suspended solids and turbidity. Suspended solids is the actual measure of the amount of sediment suspended in water column, the process 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 being influenced by the particle-size distribution, shape of particles and other dissolved materials (Sorenson *et al.*, 1977).

Studies have showed that the turbidity levels beyond natural background can affect the physiology and behavior 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 2.6.2 lists the effects of turbidity on salmonids' behavior (Bash *et al.*, 2001).

Table 2.6.2 Effects of turbidity on salmonids behavior (Bash *et al.*, 2001)

Physiological	Behavioral	Habitat
Gill Trauma	Avoidance	Reduction In Spawning Habitat
Osmoregulation	Territoriality	Effect On Hyporheic Upwelling
Blood Chemistry	Foraging And Predation	Reduction In BI Habitat
Reproduction And Growth	Homing And Migration	Damage To Redds

3. INTRODUCTION TO VIK SETTEFISK AS

3.1 Site location, water source and history

The two-month (July to august in 2014) case study was conducted at Vik Settefisk AS, a smolts farm was located in the western coast of Bergen, Norway. It is a land-based farm established in 1978, it has abundant fresh water resource from a nearby lake and it is close to sea. Salmon and rainbow trout fry in the farm were bought from Strømsnes Akvakultur AS and AquaGen AS respectively.

After many years' success since establishment, the farm suffered from water quality problem from 2008 to 2012. RAS was introduced to Vik Settefisk AS in December of 2012. Before that the main water treatment was total suspended solids removal, and production capacity was limited with many uncertainties. After employing RAS, water quality became better and more stable, in consequence the production of salmon smolts had doubled between 2011and 2013, which increased from 255 000 to 570 000.

3.2 Fish tanks and water treatment

As shown in Figure 3.1, the farm can be divided into two main parts: water treatment part and fish tanks. There were totally 11 tanks in use, and all tanks were equipped with a separate CO_2 stripper (CO_2-9000SF, Sterner). Table 3.1 shows detailed information about tanks' volume, fish species inside and status during four tests.

For sustaining the water temperature in the farm, the water treatment part was located in a heat-insulated room, Figure 3.2 shows the water flow inside and treatment process.

Reused water was filtered by a 90μm-mesh drum filter (Hydrotech, HDF 1203-2H) before they were pumped in to the water treatment part. First, water passed through a 2-meter high CO_2 stripper (CO_2-9000SF, Sterner) and then led into a closed ozone cabin. Before entering the

MBBR, reused water passed through a pH adjustment section. MBBR consisted of two chambers and has two different kinds of bio-medias (Mutag Biochip, RK Bioelements) evenly distributed inside. Fixed bed was connected with MBBR and consisted of bioblocks. Before water were pumped back to tanks, they passed another 25 µm-meshed drum filter (Hydrotech, HDF 2007-IA).

Make-up water was taken from a nearby lake at depth of around 10 meters. After passing a screen filter, they were disinfected both by ozone gas (WEDECO, GSO 50) and UV irradiation (WEDECO, B-80). Before entering the system, over-flow water was used to increase temperature through a heat exchanger (Alfa Laval, TL6-BFM).

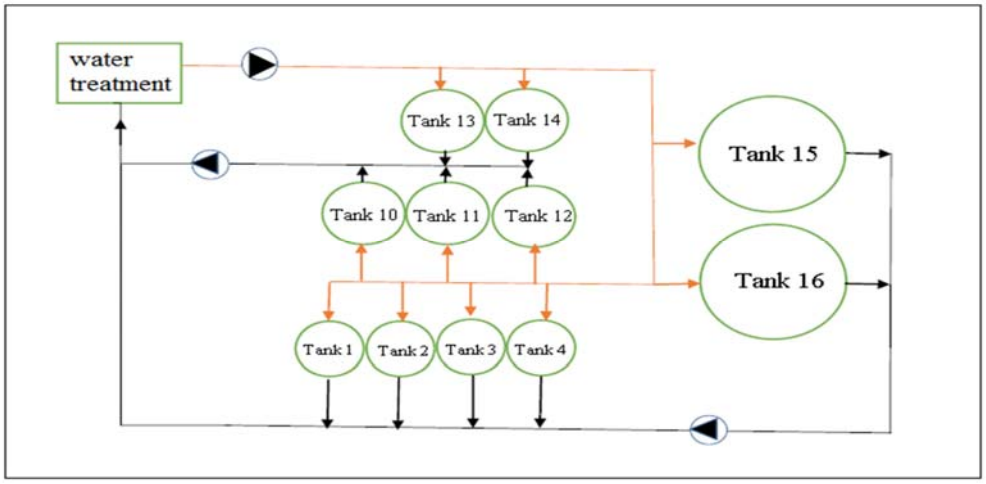

Figure 3.1 Simplified layout of the farm, fish tanks and water treatment part.

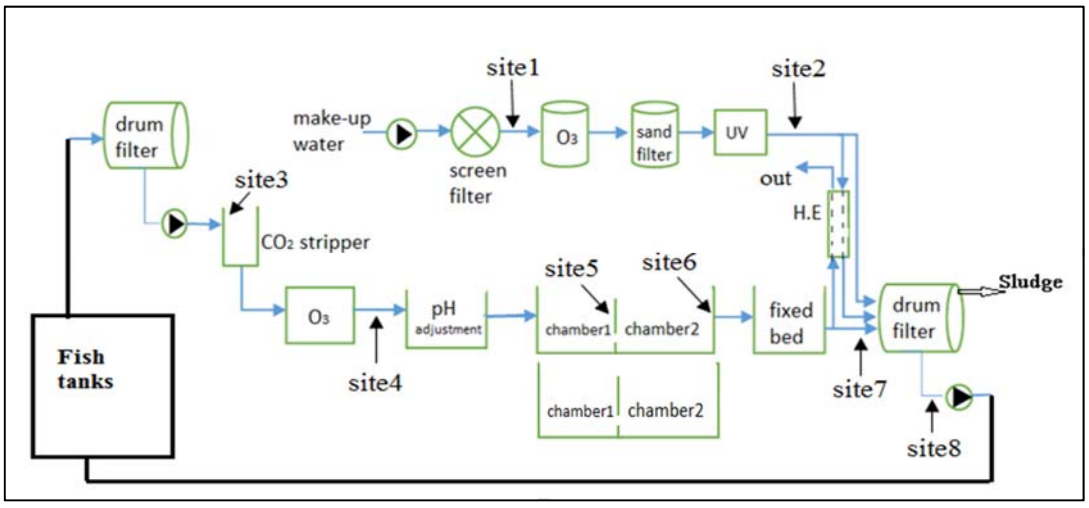

Figure 3.2 Flow chart in water treatment part and location of sampling sites.

Table 3.1 Tank volume, fish species and status during four tests.

Tank No.	Tank volume (m^3)	Indoors or outdoors	Species	Test 1 ($990m^3$)	Test 2 ($1020m^3$)	Test 3 ($420m^3$)	Test 4 ($420m^3$)
1	30	Outdoors	Fry (Salmon)	√	√	√	√
2	30	Outdoors	Fry (Salmon)	√	√	√	√
3	30	Outdoors	Fry (Salmon)	√	√	√	√
4	30	Outdoors	Fry (Salmon)	N	√	√	√
10	60	Indoors	Fry (Salmon)	√	√	√	√
11	60	Indoors	Fry (Rainbow trout)	√	√	√	√
12	60	Indoors	Fry (Rainbow trout)	√	√	√	√
13	60	Indoors	Fry (Rainbow trout)	√	√	√	√
14	60	Indoors	Fry (Rainbow trout)	√	√	√	√
15	300	Outdoors	Juvenile (Rainbow trout)	√	√	x	x
16	300	Outdoors	Juvenile (Rainbow trout)	√	√	x	x

√: in use with fresh water.

N: tank 4 was empty until 21July, when half of the fish from tank10 was transferred to tank4.

X: in use with seawater, and not accounted in the total fresh water volume.

3.3 Dimension of MBBR

Water in MBBR flowed in two parallels and passed through chamber 1 and chamber 2. Both chamber 1 and chamber 2 were 740 cm in width, 280 cm and 530 cm in length respectively. Both of them were filled up with two different types of biofilm media: RK BioElements and Mutag Biochip, in different proportion. Technical specifications and image of biofilm media are showed in Table 3.2 and Figure 3.3, respectively.

Table 3.2 Technical specifications of RK BioElements and Mutag Biochip.

Parameters	RK BioElements (Medium)	Mutag Biochip
Volume weight(kg/m^3)	172	170
Number (pcs/m^3)	255 000	
Specific surface area (m^2/m^3)	750	3000
Density (g/cm^3)	1.0	<1.0
Material	PP	PE
Shape	Round，cylinder	Round，paraboloid

Figure 3.3 Image of Mutag Biochip (left) and RK BioElements Medium.

In chamber 1, the depth of biofilm media is 65cm (when the chamber is drained of water), and has the volume of 13.468 m^3. While RK BioElements accounts for 75% in volume and the rest 26% is Mutag Biochip. Therefore, the total protected surface area in chamber 1 is 17 677 m^2.

In chamber 2, the depth of biofilm media is 76cm (when the chamber is drained of water), and has the volume of 29.807 m^3. While RK BioElements accounts for 46% in volume and the rest 54% is Mutag Biochip. Therefore, the total protected surface area in chamber 1 is 58 571 m^2.

The water level in the MBBR was maintained around 180cm. In operation, when biofilm medias are immersed with water, the actual water volume is about 73.7%. Detailed information about chamber 1 and chamber 2 are summarized in Table 3.3.

Table3.3 Detailed information about chamber1 and chamber 2.

Chamber	L*W*H (cm)	Water level(cm)	Water volume(L)	Biomedia level(cm)	Biomedia volume(L)	% of media	Protected surface area m^2
Chamber1	280*740*200	180	27487	65	13468	49.0	17 677
Chamber2	530*740*200	180	52029	76	29807	57.3	58 571

3.4 Sampling sites and measured parameters

All the 8 sampling sites were located in water treatment part, they were marked in Figure 3.2 and measured parameters at different sites are listed in Table 3.4. The exact sampling sites are mentioned below.

Site 1: make-up water after screen filter, before ozone treatment,

Site 2: make-up water after treated by UV irradiation,

Site 3: reused water after drum filter, before CO_2 stripper,

Site 4: reused water after the closed ozone cabin,

Site 5: reused water between the two bio-media chambers,

Site 6: reused water before fixed bed,

Site 7: reused water before drum filter,

Site 8: reused water after drum filter.

Table 3.4 Measured parameters at different sampling sites.

Site	1	2	3	4	5	6	7	8
NH_4-N			√	√	√	√	√	
NO_2-N			√	√	√	√	√	
NO_3-N			√	√	√	√	√	
COD			√	√	√	√		√
Turbidity			√	√				√
TSS						√	√	√
Temp.	√		√					
ALK.	√		√				√	
pH	√		√				√	
Bacteria	√	√	√	√				

4. MATERIALS AND METHODS

This case study was carried out at the smolts farm of Vik Settefisk AS (Bergen). Detailed information has been mentioned in Section 3, Introduction to Vik Settefisk AS. In total, four tests has been conducted during the case study, and labelled as test 1, test 2, test 3 and test 4 respectively.

4.1 Fish size, daily feeds amount and tank volume

There were totally 11 tanks in use, their layout were showed in Figure 3.1. The detailed information about tank volume, species and status during four tests were showed in Table 3.1. There were two species reared in the farm, rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Salmon fry in Tank 1-4 and 10 were bought from Strømsnes Akvakultur AS, and rainbow trout fry in Tank 11-14 were bought from AquaGen AS. In Tank 15 and 16 were rainbow trout weighted around 200g, and were ready to smoltification.

All the feeds used were from Biomar Company. Table 4.1.1 shows the feed types and diameter. Feeds were distributed evenly by screw feeder (Betten feeders, Betten Maskinstasjon AS, Norway) and feed rate was adjusted every week according to average fish weight and water temperature. Average weight (g) of fish and daily feed amount (kg) in each tank during four tests are showed in Table 4.1.2 and Table 4.1.3 respectively.

Table 4.1.1 Feed type in each tank.

Tank	Feed type	Feed diameter (mm)
1-4	INICIO Plus	1.5
10-14	Intro 15 Svev	2.0
15-16	Intro 100 Svev	4.5

Table 4.1.2 Average weight (g) of fish in each tank during four tests.

Tank NO.	Species	Test 1	Test 2	Test 3	Test 4
tank 1	Salmon	24.89	28.58	31.55	40.20
tank 2	Salmon	28.96	34.15	38.28	46.21
tank 3	Salmon	31.66	37.15	40.98	28.24
tank 4	Salmon	a	37.55	41.83	18.40
tank 10	Salmon	34.88	40.82	45.87	29.39
tank 11	Rainbow Trout	26.74	33.60	38.36	40.81
tank 12	Rainbow Trout	26.74	34.01	38.85	55.51
tank 13	Rainbow Trout	26.74	33.60	38.44	59.11
tank 14	Rainbow Trout	26.75	33.61	38.46	42.31
tank 15	Rainbow Trout	189.13	238.60	256.30	272.69
tank 16	Rainbow Trout	172.89	238.18	255.14	270.86

a: Tank 4 was empty until 21July, when half of the fish from tank 10 was transferred to tank 4.

Table 4.1.3 Daily feed amount (kg) in each tank during four tests.

Feed(KG)	Type	Test1	Test2	Test3	Test4
tank 1	Salmon	30	30	30	50
tank 2	Salmon	25	30	30	30
tank 3	Salmon	50	30	30	30
tank 4	Salmon		30	30	25
tank 10	Salmon	50	75	75	50
tank 11	Rainbow Trout	50	75	50	50
tank 12	Rainbow Trout	50	75	50	50
tank 13	Rainbow Trout	50	75	50	50
tank 14	Rainbow Trout	50	75	50	50
tank 15	Rainbow Trout	250	200	220	220
tank 16	Rainbow Trout	250	200	220	220
Total(KG/Day)		855	895	395[a]	385[a]
FLR(kg/m^3)		4.53	1.70	2.16	38.19

a: Feeds amount in tank 15 and 16 are not included, because sea water were introduced after test2 and these two tanks were separated from the fresh water reuse system.

FLR: Feed Loading Rate (kg/m^3) = kg feed/ m^3 make-up water

4.2 Make-up water, recirculating rate and retention time

To compensate for the water loss and for water temperature adjustment purpose, make-up water was taken from a nearby lake at depth of around 10 meters. The make-up water had stable quality: temperature around 10℃, pH ranged from 5.9 to 6.1 and a low alkalinity level around 5mg/L (as $CaCO_3$).

After passing a screen filter, they were disinfected by both ozone gas (WEDECO, GSO 50) and UV irradiation (WEDECO, B-80). Before entering the system, over-flow water was used to increase temperature through a heat exchanger (Alfa Laval, TL6-BFM).

In test 1 and test 2, the fresh water flow rate was 7000 L/min; while in test 3 and test 4, seawater had been introduced to tank 15 and tank 16, so the fresh water flow in the system was reduced to 5000 L/min. Table 4.2 shows the make-up water flow, recirculating rate and retention during the study.

Table 4.2 Make-up water flow, total fresh water flow, recirculating rate, and retention time in chamber 1 and chamber 2 during four tests.

Test	Make-up water flow (L/min)	Total fresh water flow (L/min)	Recirculating rate (%)	Retention time in chamber 1 (min)	Retention time in chamber 2 (min)
1	131	7000	98.13%	3.93	7.43
2	365	7000	94.79%	3.93	7.43
3	127	5000	97.46%	5.50	10.41
4	7	5000	99.86%	5.50	10.41

4. 3 UV and ozone dosage

UV (Wedeco GmbH, B-80, Herford, Germany) was used to disinfect make-up water. Table 4.3.1 shows technical information of the equipment.

Table 4.3.1 Technical information of UV instrument (WEDECO B-80).

Parameters	
Characteristic	Stainless steel reactor with multiple UV lamps
Wave length, nm	254
B x H x T (mm)	1,295 x 430 x 270
UV Dose(w/m^2)	300 (at the end of lamp lifetime)
UV transmission	98% (at end of lamp lifetime)
Application	Drinking water; Process water; Warm water
Capacity	Up to 600 m^3/h

During the experimental period, output of the UV light was 92.0 W/m^2. The chamber for irradiation is 51 L, and retention time differs depends on water flow. Table 4.3.2 shows UV dosage in make-up water flow during four tests.

Table 4.3.2 UV dosage in make-up water flow.

Test	Output W/m^2	Water flow(L/min)	Retention time(min)	UV dosage mJ/cm^2
Test 1	92.0	78	0.65	358.8
Test 2	92.0	365	0.14	77.28
Test 3	92.0	127	0.40	220.8
Test 4	92.0	7	7.29	4024

Pure oxygen was used to generate ozone onsite (Wedeco GmbH, GSO 50, Herford, Germany). The amount of ozone generated per hour (g/h) can be calculated according to the following equation:

$$m_{o_3} = A \times V_{max} \times C_{o_3}$$

m_{o_3} : The ozone quantity generated per hour (g/h),

A: power consumption on display (%),

V_{max} : The maximum feed oxygen flow (5.7 m^3/h for GSO 50 generator),

C_{o_3}: Concentration of generated ozone (g/m^3).

In test 1 and 2, the ozone generator operated at 95% capacity (A=0.95). In test 3 and 4, the generator operated at 60 % capacity (A=0.6). According to performance curve of the ozone generator (Figure 4.3.1), in test 1 and 2 the concentration of generated ozone was 80 g/m^3; while in test 3 and 4, the concentration of generated ozone was 58 g/m^3.

Ozone were distributed to disinfect both make-up water and reused water at different percentage. Table 4.3.3 shows calculated ozone dosage in make-up and reused water.

Table 4.3.3 Ozone dosage in make-up water and reused water.

	Make-up water				Reused water			
	Flow rate, L/min	Retention time, min	C_{O3}, mg/L	Ozone C*t, Min*mg/L	Flow rate, L/min	Retention time, min	C_{O3}, mg/L	Ozone C*t, Min*mg/L
Test 1	78	16.15	10	161.46	7000	3.43	0.97	3.34
Test 2	365	3.45	2.19	7.56	7000	3.43	0.97	3.34
Test 3	127	9.92	6.93	68.73	5000	4.8	0.93	4.44
Test 4	7	180	108	19542	5000	4.8	0.95	4.56

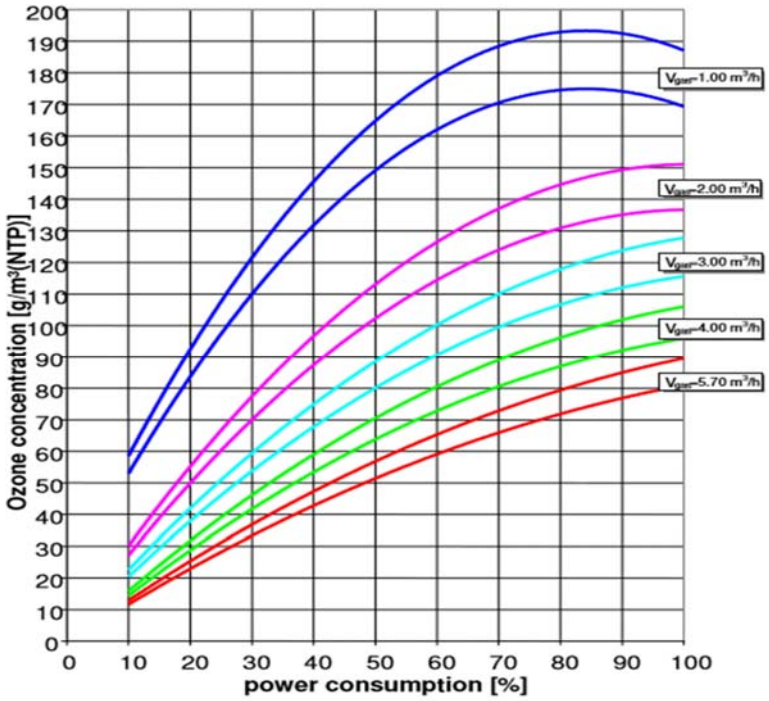

Figure 4.3 Performance curve (V_{gas}=5.70m^3/h) of the ozone generator (Operation Instruction of EFFIZON Ozone Generator, GSO-50).

4. 4 Analysis of water quality

Water sample (500mL) was collected at depth of 50 cm at sampling sites (Figure 3.2), and stored in polyethylene (PE) bottle for analysis. Parameters like dissolved oxygen, temperature and pH were measured on site. Water sample was first used to measure heterotrophic bacteria count and their turbidity, later the concentration of NH_4-N, NO_2-N, NO_3-N and COD measured, and in the end alkalinity and total suspended solids.

4.4.1Measurement of dissolved oxygen, temperature, pH

Dissolved oxygen, temperature were measured directly at sampling sites by a portable meter, OxyGuard Handy Polaris 2 (OxyGuard International AS, Birkerød, Denmark). Dissolved oxygen concentration are shown both in mg/L (or ppm) and in saturation (%), and temperature is showed in degree Celsius (°C). pH was measured at each sampling site directly by portable pH meter (OxyGuard Handy pH, Farum, Denmark).

4.4.2 Measurement of NH_4-N, NO_2-N, NO_3-N and COD

Spectroquant® Photometer NOVA 60(Merck KGaA, Darmstadt, Germany) (Figure 4.4.1) was used to determine the concentration of NH_4-N, NO_2- N, NO_3- N and COD (mg/L). The first three parameters were measured in a similar procedure (see Appendix 1-3), but using different test kits.

In COD concentration measurement, thermoreactor (CR3200, Brannum Lane, Yellow Springs, USA) was used. Pretreated water samples were incubated at 148 °C for 120 min in the equipment. Detailed measurement procedures are listed in Appendix 4, and Table 4.4.1 shows the characteristic quality data of the method.

Figure 4.4 Schematic diagram of Spectroquant® Photometer NOVA 60.

Table 4.4 Characteristic quality data of each parameter.

Parameters	NH_4-N	NO_2- N	NO_3- N	COD
Cell(mm)	10	10	10	10
Standard deviation (mg/L)	±0.023	±0.008	±0.11	±0.29
Co-efficiency of variation (%)	±1.5	±1.4	±0.85	±1.4
Co-efficiency interval (mg/L)	±0.06	±0.02	±0.3	±0.7
Number of lots	40	48	20	52
Measuring range (mg/L)	0.05-3.00	0.02-1.00	1.0-25	4.0-40.0
Accuracy of the measured value (mg/L)	max.±0.08	max.±0.03	max.±0.5	max.±1.5
Dilution times	4-8	4	4	2

4.4.3 Measurement of Alkalinity

Alkalinity was measured by titration 100mL water sample with hydrochloric acid (HCl, 0.1 M) to the methyl orange endpoint (pH of 4.5). Then alkalinity is calculated by equation below:

$$Alkalinity = \frac{V_1 \times C_1 \times 50}{V_2}$$

Where: V_1 is amount of hydrochloric acid used to reach pH 4.5 (mL)

C_1 is the concentration of acid (mole/L)

V_2 is the volume of water sample (mL)

Alkalinity is express in mg/L (as $CaCO_3$).

4.4.4 Measurement of total suspended solids (TSS) and turbidity

TSS was measured by filtering well-mixed water sample through a weighted glass fiber filter (0.45 µm, Whatman, GF/C), and then the filter was dried at 105°C. The weight increase of the filter divided by the volume of water filtered is the concentration of total suspended solids; it is expressed in mg/L. Turbidity was measured by nephelometer (Merck turbiquant 3000 IR), it is expressed in NTU.

All analyzer instruments were calibrated before using.

4.4.5 Measurement of heterotrophic bacteria load

The heterotrophic bacteria load in terms of detection and enumeration was measured by a ready to use, rehydrated plate with indicator (Compact Dry AQ, Uffing, Germany).

At first 1 mL water sample was dropped in the middle of the plate, and then the water sample was diffused into it and evenly spread on the plate, and then transformed the rehydrated plate into a gel within seconds. After that put a cap on the plate and turned it over, then put it in an incubator (at 36±2°C for 44±4h) in a horizontal position. After incubation, counted the number of all grown colonies underneath the plate.

4.5 Statistical model

Results expressed in an average with standard deviation of three replicates. Statistical analysis done by one-way ANOVA and statistical difference was considered to be significant if $p < 0.05$.

4.5.1 Calculation of TAN concentration from NH_4-N concentration

As mentioned in literature review part, TAN is the sum of NH_4-N and NH_3-N, and the ratio between NH_4-N and NH_3-N depends on temperature, salinity and pH. Based on NH_4-N concentration, TAN concentration can be calculated by equation below:

$$C_{TAN} = \frac{C_{NH4-N}}{1 - P_{NH3-N}}$$

Where C_{TAN} is TAN concentration (mg/L),

C_{NH4-N} is measured NH_4-N concentration (mg/L),

P_{NH3-N} is the percent of NH_3-N in TAN at different temperature and pH.

4.5.2 Calculation of areal TAN removal (ATR) rate

$$ATR = \frac{Kc \times (TAN_{(a)} - TAN_{(b)}) \times Q}{A}$$

ATR is expressed in g/m^2.d, which means g TAN removed per m^2 surface area of bio-media per day. Where Kc is the unit conversion constant (24h*60min/1000). $TAN_{(a)}$-$TAN_{(b)}$ means the TAN concentration difference (mg/L) between site a and site b. Q is the water flow rate in the system (L/min). A is the protected surface area of bio-medias (m^2).

4.5.3 Calculation of areal nitrite removal (ANR) rate

$$ANR = ATR + \frac{Kc \times (NO2.N_{(a)} - NO2.N_{(b)}) \times Q}{A}$$

ANR is expressed in g/m^2.d, which means g NO_2-N removed per m^2 surface area of bio-media per day. Where Kc is the unit conversion constant (24h*60min/1000). NO_2-$N_{(a)}$ - NO_2-$N_{(b)}$ means the NO_2-N concentration difference (mg/L) between site a and site b. Q is the water flow rate in the system (L/min). A is the protected surface area of bio-medias (m^2).

5. RESULTS

5.1 Temperature, pH, dissolved oxygen and alkalinity variation in make-up and reused water

As shown in Table 5.1, make-up water had stable water quality. With Temperature around 9.20-10.8℃, pH varied from 5.67 to 6.13, dissolved oxygen (DO) ranged from 7.0 to 12.7mg/L, and a low alkalinity (5.0 mg/L as CaCO3).

In reused water, temperature was around 13.90-20.80℃, pH varied from 6.91 to 7.34, DO ranged from 5.90 to 11.60mg/L, and alkalinity ranged 80.0-147.5mg /L. It was worth noting that water temperature in test 1 (18.10-20.80 ℃) was higher than in the other tests.

Table 5.1 Temperature, pH, DO and alkalinity variation in make-up and reused water.

	Reused water				Make-up water(Lake)			
	T (℃)	pH	DO(mg/L)	Alka(mg/L)	*T* (℃)	pH	DO(mg/L)	Alka(mg/L)
Test 1	18.10-20.80	6.91-7.22	6.20-11.60	107.5-112.5	9.20-9.39	5.80-6.13	/	5.0
Test 2	16.10-16.90	6.95-7.11	6.10-11.20	107.5-130.0	10.3-10.7	5.73-5.90	/	5.0
Test 3	13.90-14.30	6.94-7.23	7.10-11.00	80.0-90.0	9.9-10.8	5.67-5.97	7.2-8.6	5.0
Test 4	15.90-16.20	7.06-7.34	5.90-10.80	132.5-147.5	10.4-10.5	5.67-5.93	7.0-12.7	5.0

5.2 Nitrogenous waste concentration and removal rate

5.2.1 TAN, free ammonia concentration and Areal TAN Removal (ATR) rate

As shown in Table 5.2.1, highest TAN concentration was observed in test 2, which was 16.32±0.17 mg/L at site 3 (before CO_2 stripper) and 14.68±0.47 mg/L at site 7 (before drum filter) respectively. TAN concentration in test 3 and 4 was lower compared with test 1 and 2. There was significant decline in TAN concentration between site 3 and 7 in all tests ($P<0.05$).

Similar to TAN concentration, highest free ammonia concentration was observed in test 2, with lowest value of 0.0547±0.0017 mg/L at site 7 (before drum filter) (Figure 5.2.1). In the other tests, free ammonia concentration was well below 0.01 mg/L at site 7. In all tests, free ammonia concentration declined significantly between site 3 and 7 ($P<0.05$).

Table 5.2.1 TAN concentration (mg/L) at different sites during four tests (M±SD. Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum filter).

n=3	Site 3	Site 4	Site 5	Site 6	Site 7
Test 1	3.63±0.05	2.99±0.09	2.68±0.08	1.57±0.00	1.27±0.08
Test 2	16.32±0.17	15.88±0.08	15.11±0.62	14.68±0.26	14.68±0.47
Test 3	1.44±0.23	1.45±0.06	1.10±0.05	0.49±0.02	0.49±0.02
Test 4	2.31±0.02	1.88±0.05	1.30±0.11	0.52±0.03	0.58±0.12

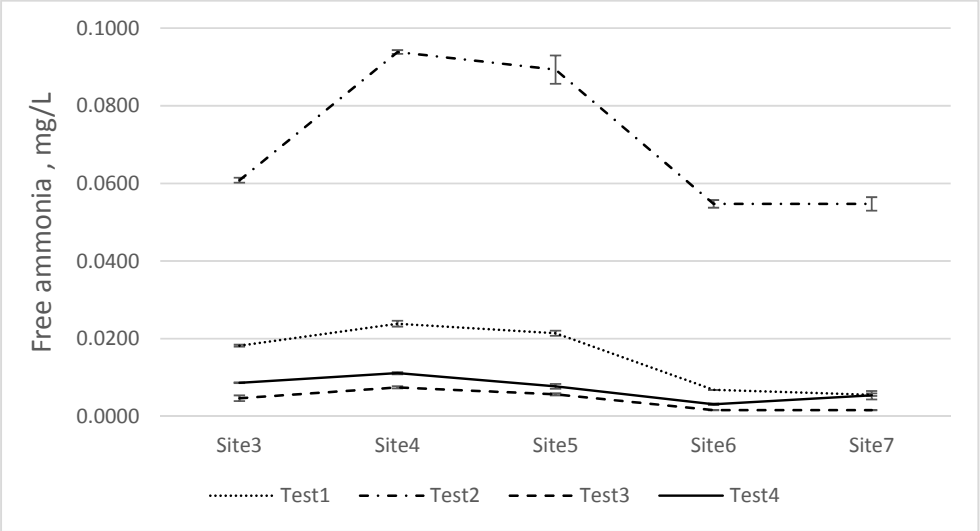

Figure 5.2.1 Free ammonia concentration (mg/L) variation at different sites during four tests (M ±SD. Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum filter, n=3).

Table 5.2.2 Areal TAN removal rate (g/m^2.d) in MBBR during four tests (M ±SD. Chamber 1: site 4-5, Chamber 2: site 5-6. Site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR).

n=3	Chamber 1	Chamber 2	Total, MBBR
Test 1	0.176 ± 0.022	0.193 ± 0.014	0.369 ± 0.027
Test 2	0.439 ± 0.329	0.074 ± 0.143	0.513 ± 0.186
Test 3	0.142 ± 0.034	0.074 ± 0.007	0.216 ± 0.027
Test 4	0.235 ± 0.062	0.096 ± 0.013	0.330 ± 0.049

Together with highest free ammonia and TAN concentration, the highest areal TAN removal (ATR) rate was also observed in test 2 (Table 5.2.2), which was 0.513±0.186 g/m^2.d. In test 1, chamber 1 and 2 had similar efficiency. While in test 3 and 4, chamber 1 showed higher efficiency than chamber 2. As shown in Figure 5.2.2, increased average TAN concentration resulted in a higher ATR rate.

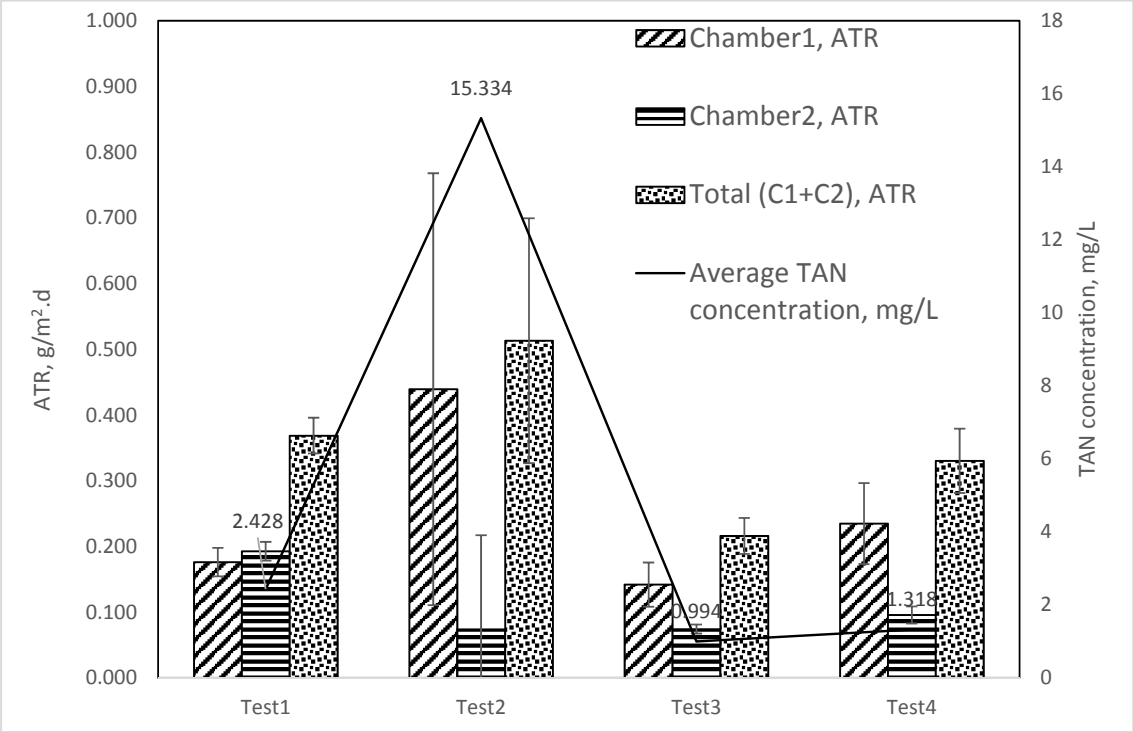

Figure 5.2.2 Areal TAN removal rate (g/m^2.d) in chamber1 and chamber2 during four tests (Chamber 1: site 4-5, Chamber 2: site 5-6. Site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR).

As shown in Table 5.2.3, constant reduction in TAN concentration was only observed in test 1. In test 2, TAN reduction percent was low due to high initial TAN concentration (16.32±0.17 mg/L at site 3). It is worth noting that except in test 3, TAN concentration showed reduction between site 3 and 4 (water passed through CO_2 stripper and closed ozone cabin). In addition, chamber 2 (S5-S6) had higher TAN reduction percent than chamber 1 (S4-S5), except in test 2 when TAN reduction percent were low in both chambers.

Table 5.2.3 TAN reduction percent (%) between each site（M ±SD. Site 3:before CO_2 stripper; site 4:after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum filter）.

n=3	S3-S4	S4-S5	S5-S6	S6-S7
Test 1	17.52±1.93	10.30±1.12	41.62±1.81	18.80±4.83
Test 2	2.68±1.50	4.86±3.63	2.62±5.46	-0.01±2.98
Test 3	-1.94±11.27	23.93±4.98	54.84±2.84	-0.21±6.54
Test 4	18.91±2.71	30.53±7.45	59.58±3.71	-9.62±15.15

5.2.2 NO_2-N concentration and areal nitrite removal (ANR) rate

As shown in Table 5.2.4, the highest NO_2-N concentration was observed in test 1, which were 1.80±0.07 mg/L at site 3 (before CO_2 stripper) and 1.59±0.08 mg/L at site 7 (before drum filter) . The lowest NO_2-N concentration was observed in test 3, which were 0.45±0.02 mg/L at site 3 and 0.36±0.00 mg/L at site 7. Similar to TAN concentration variation (Table 5.2.1), NO_2-N concentration in test 3 and 4 were lower than in test 1 and 2. In addition, there was significant decline in NO_2-N concentration between site 3 and 7 in all tests ($P<0.05$).

Table 5.2.4 NO_2-N concentration (mg/L) at different sites during four tests (M±SD. Site3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum filter).

n=3	Site 3	Site 4	Site 5	Site 6	Site 7
Test 1	1.80±0.07	1.61±0.02	1.47±0.11	1.49±0.05	1.59±0.08
Test 2	1.20±0.03	1.12±0.03	1.09±0.02	1.03±0.04	1.08±0.03
Test 3	0.45±0.02	0.45±0.05	0.40±0.03	0.35±0.02	0.36±0.00
Test 4	0.75±0.02	0.71±0.02	0.73±0.02	0.57±0.02	0.65±0.02

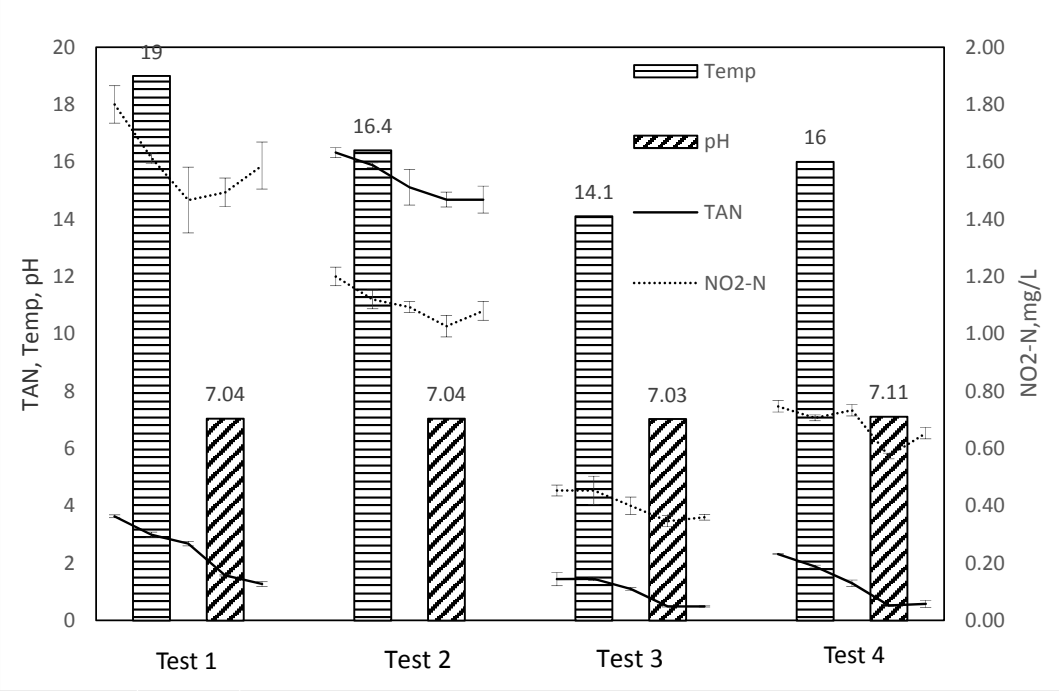

Figure 5.2.3 TAN and NO_2-N concentration (mg/L) variation from site 3 to site7 in four tests (Site3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum filter).

Table 5.2.5 Areal nitrite removal (ANR) rate (g/m^2.d) in MBBR (M ±SD. Chamber 1: site 4-5, Chamber 2: site 5-6. Site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR).

n=3	Chamber 1	Chamber 2	Total, MBBR
Test 1	0.26 ± 0.08	0.19 ± 0.03	0.45 ± 0.06
Test 2	0.45 ± 0.31	0.09 ± 0.15	0.54 ± 0.17
Test 3	0.16 ± 0.01	0.08 ± 0.00	0.24 ± 0.01
Test 4	0.22 ± 0.06	0.12 ± 0.01	0.34 ± 0.04

Areal nitrite removal (ANR) rate ranged from 0.24±0.01 to 0.54±0.17 g/m^2.d (Table 5.2.5). The highest value was observed in test 2, and the lowest in the test 3. In general, chamber 1 showed higher average ANR rate than chamber 2 (Figure 5.2.4).

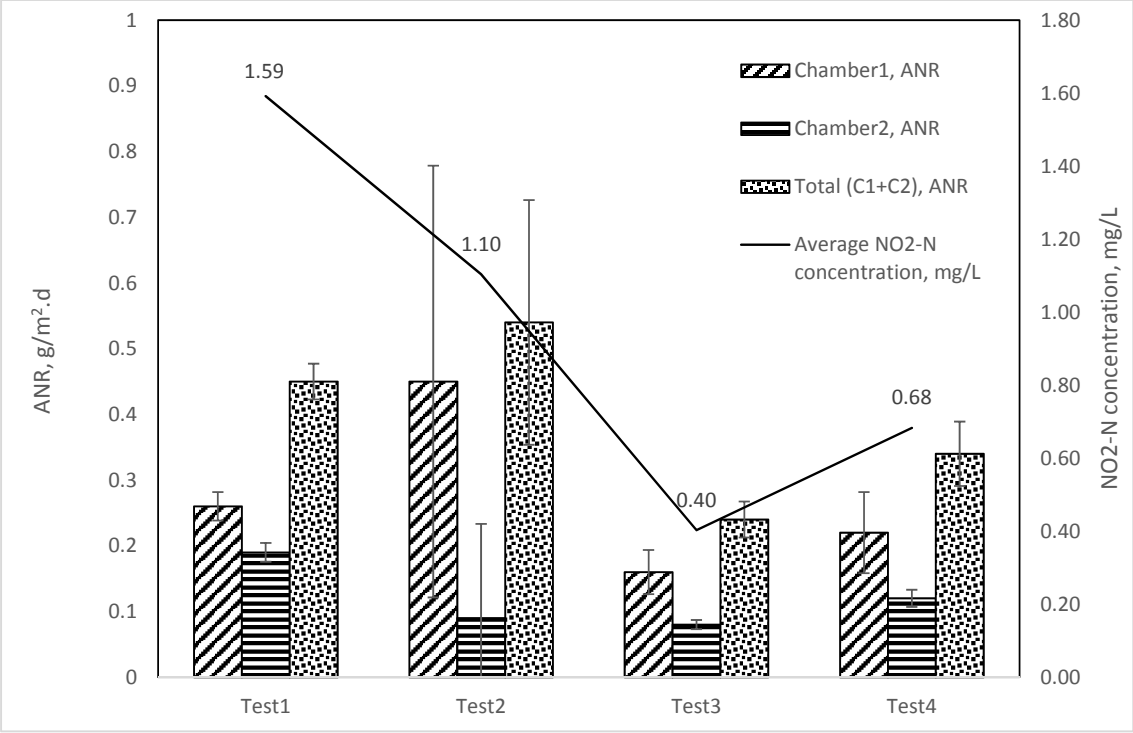

Figure 5.2.4 Areal nitrite removal rate (g/m^2.d) in chamber 1 and chamber 2 during four tests (Chamber 1: site 4-5, Chamber 2: site 5-6. Site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR).

5.2.3 NO_3-N concentration variation and relationship with feed loading rate

The lowest NO_3-N concentration was observed in test 3, which ranged from 23.07±1.24 to 35.20±1.42 mg/L (Table 5.2.6). Test 4 showed the highest NO_3-N concentration and ranged from 49.60±2.94 to 62.40±8.03 mg/L. As mentioned above, TAN concentration declined significant between site 3 and 7 in all tests ($P<0.05$). However in test 2 and 3, NO_3-N concentration showed significant increase between site 3 and 7 ($P<0.05$). While in test 1 and 4, NO_3-N level showed decline in average value.

Table 5.2.6 NO_3-N concentration (mg/L) at different sites during four tests (M±SD. Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum filter).

n=3	Site 3	Site 4	Site 5	Site 6	Site 7
Test 1	53.60±10.37	48.93±3.95	45.73±5.62	46.13±2.45	46.27±1.68
Test 2	41.33±1.24	47.60±5.67	50.13±4.26	48.40±1.18	49.73±1.61
Test 3	23.07±1.24	36.93±1.80	34.80±1.13	38.67±4.74	35.20±1.42
Test 4	62.40±8.03	58.67±10.66	49.60±2.94	59.87±7.84	59.13±8.20

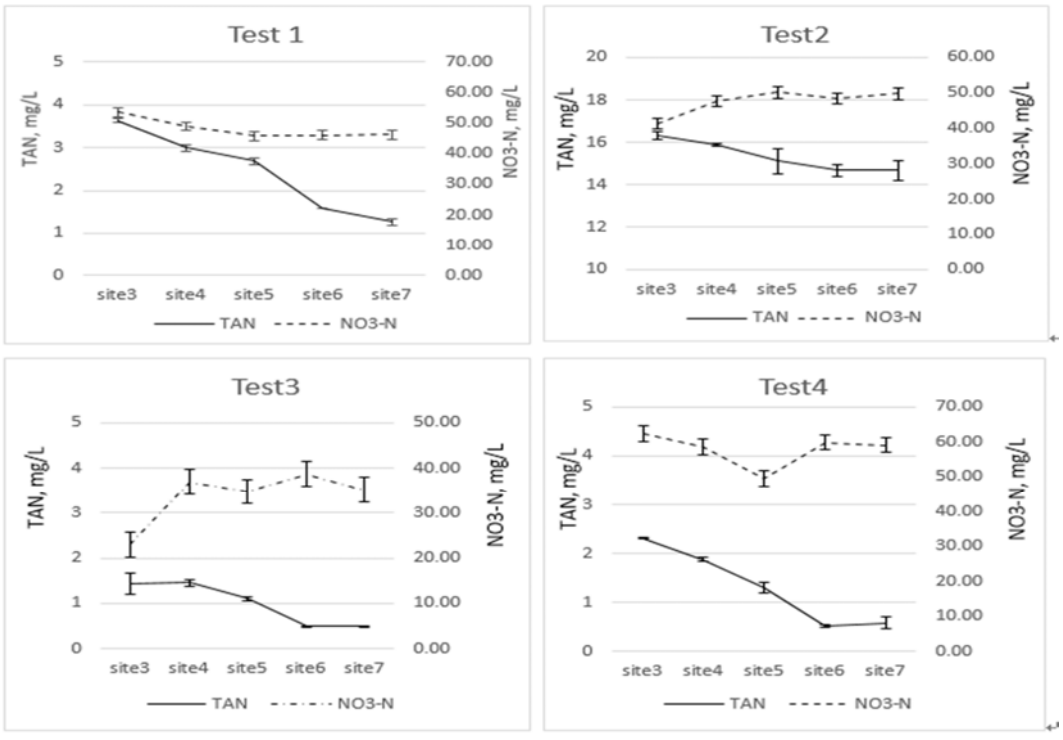

Figure 5.2.5 Variation of TAN and NO_3-N concentration (mg/L) in each test (Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum).

As shown in Figure 5.2.6, except in test 4, average NO_3-N concentration showed negative correlation with feed loading rate. When the feed loading rate was from 1.70 to 4.53 kg feed/ m^3 make up water in the first three tests, NO_3-N concentration ranged from 23.07±1.24 to 53.60±10.37 mg/L. However, when the feed loading rate was at 38.19 kg feed/ m^3 make up water in test 4, NO_3-N concentration ranged from 49.60±2.94 to 62.40±8.03 mg/L. The result indicated that there was no accumulation of NO_3-N at a high feed loading rate.

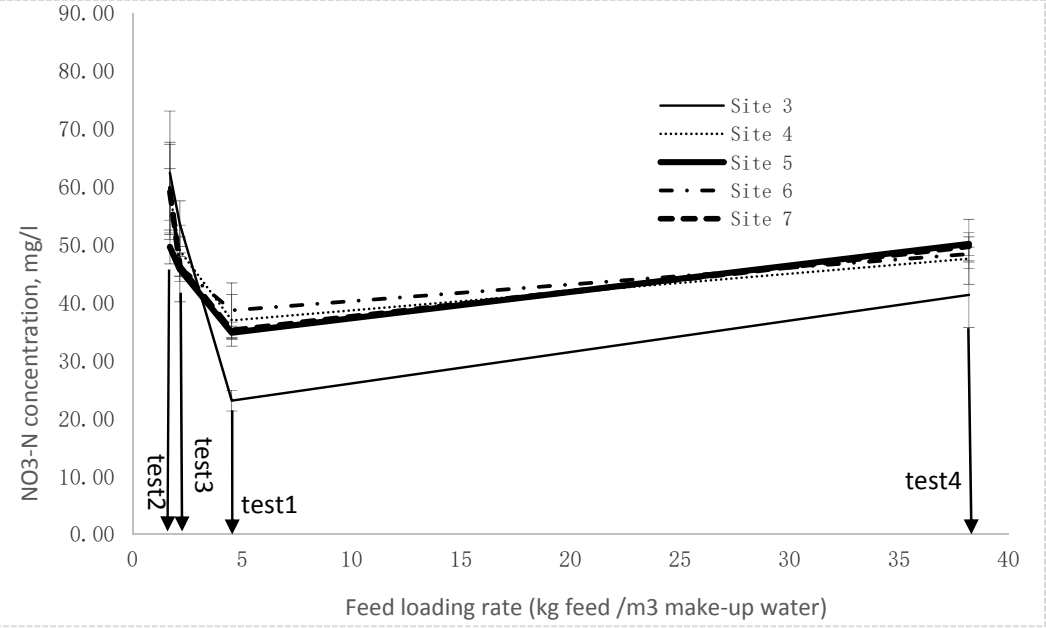

Figure 5.2.6 Relationship between feed loading rate (FLR) and NO_3-N concentration variation in four tests (n = 3. Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 7: before drum filter).

5.3 COD concentration and removal rate, COD/TAN ratio and TAN reduction (%)

COD concentration in test 3 and 4 were lower than in test 1 and 2 (Figure 5.3.1).The lowest COD concentration was observed in test 3, which ranged from 36.73±0.25 to 40.73±1.76 mg/L. Except in test 1, there was significant decline in COD concentration between site 3 and site 8 ($P<0.05$).

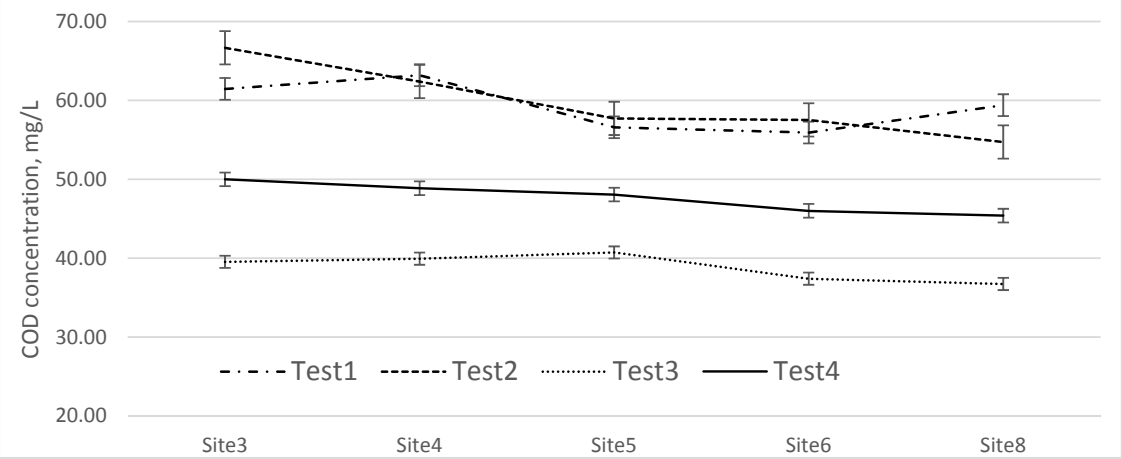

Figure 5.3.1 COD concentration (mg/L) at different sites during four tests (Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR; site 8: after drum filter)

5.3.1 Areal COD removal rate in MBBR

Together with lowest COD concentration, the lowest areal COD removal rate (0.08±0.74 g/m^2.d) was also observed in test 3 (Table 5.3.1). The highest areal COD removal rate was observed in test 1, which was 3.88±0.80 g/m^2.d. Test 1 and 2 showed higher areal COD removal rate than in test 3 and 4. In test 1, chamber 1 was more efficient in areal COD removal rate than chamber 2. In test 4, chamber 1 and 2 had similar areal COD removal rate (Figure 5.3.2).

Table 5.3.1 Areal COD removal rate (g/m^2.d) in MBBR (M±SD. Chamber 1: site 4-5, Chamber 2: site 5-6. Site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR).

n=3	Chamber 1	Chamber 2	Total, MBBR
Test 1	3.76 ± 0.99	0.11 ± 0.18	3.88 ± 0.80
Test 2	2.66 ± 2.60	0.03 ± 0.20	2.70 ± 2.42
Test 3	-0.33 ± 0.98	0.41 ± 0.27	0.08 ± 0.74
Test 4	0.33 ± 0.18	0.25 ± 0.07	0.58 ± 0.13

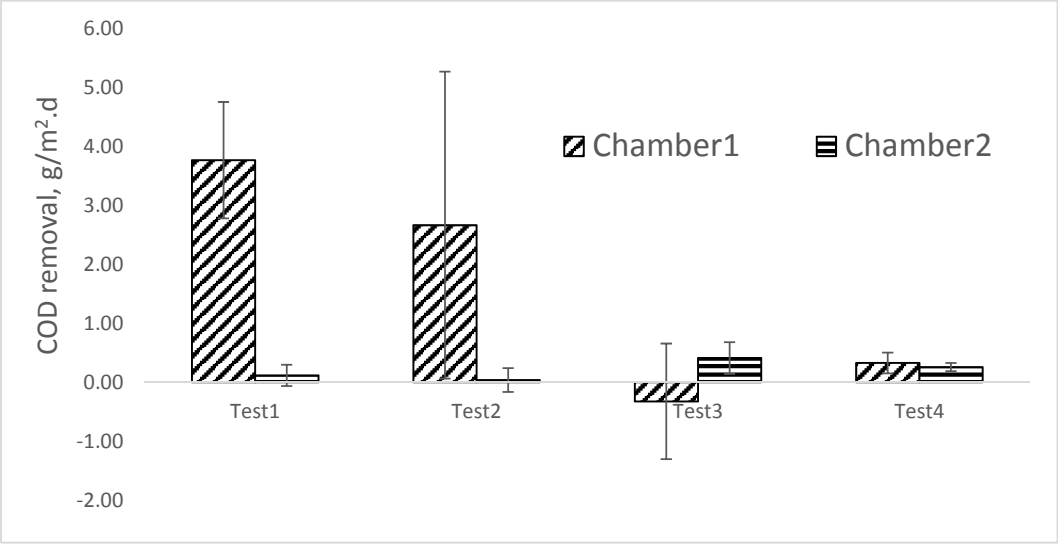

Figure 5.3.2 Areal COD removal rate (g/m^2.d) in chamber 1 and 2 during four tests (Chamber 1: site 4-5. Chamber 2: site 5-6. Site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR).

5.3.2 COD/TAN ration and TAN reduction (%)

As shown in Table 5.3.2, test 2 had a low and stable COD/TAN ratio, which decreased from 4.09±0.02 at site 3 (before CO_2 stripper) to 3.92±0.03 at site 6(after MBBR). This was mainly due to the high initial TAN level (Table 5.2.1) and low TAN reduction percent (Table 5.2.3) in the test. However, COD/TAN ratio surged from site 3 to 6 in the other tests, especially between site 5 and 6. In test 3 and 4, COD/TAN ratio doubled from site 5 to 6 and reached 75.73±3.32 and 88.35±5.08 respectively. The main reason was high TAN reduction percent (more than 50 %) in these two tests (Table 5.2.3). In test 1, COD/TAN ratio increased from 21.11±0.92 to 35.73±0.22 from site 5 to 6, with a 41.62±1.81 % TAN reduction between site 5 and 6.

Table 5.3.2 COD/TAN ratio at different sites during four tests (M ± SD. Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR).

n = 3	Site 3	Site 4	Site 5	Site 6
Test 1	16.94±0.31	21.13±0.56	21.11±0.92	35.73±0.22
Test 2	4.09±0.02	3.93±0.24	3.83±0.23	3.92±0.03
Test 3	28.08± 4.42	27.64±0.56	37.11±0.80	75.73±3.32
Test 4	21.61±0.07	26.08±0.90	37.22±2.87	88.35±5.08

The highest areal TAN removal rate (0.513±0.186 g/m^2.d) rate was observed in test 2 at a low COD/TAN ratio (ranged from 3.92±0.03 to 4.09±0.02). At high COD/TAN ratio in test 3 and 4, areal TAN removal rate was much lower than in test 1. The result indicated that a low COD/TAN ratio could increase areal TAN removal rate.

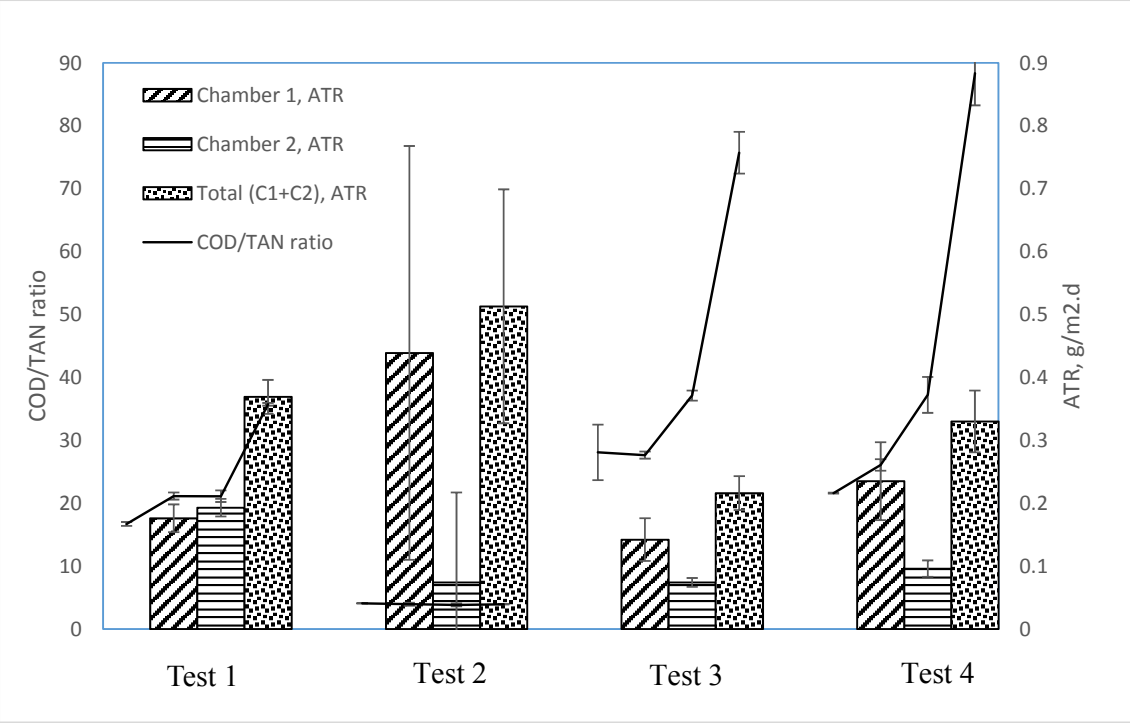

Figure 5.3.3 COD/TAN ratio (from site 3 to site 6) and ATR rate variation during four tests (Site 3: before CO_2 stripper; site 4: after ozone cabin; site 5: between MBBR; site 6: after MBBR. Chamber 1: site 4-5. Chamber 2: site 5-6).

5.4 Total Suspended solids (TSS) and its relationship with COD concentration

There was no significant difference in TSS concentration between each site in one test, but TSS showed decline on average value in this study (Figure 5.4.1). In addition, TSS concentration in test 3 and 4 was lower than in test1 and 2 on average value. It coincided with the difference in feed amount, in test 3 and 4 the feed amount was less than half of that in test 1 and 2.

As shown in Figure 5.4.2, COD concentration can be expressed by TSS in a linear model with equation COD=0.9586×TSS+35.188 (R^2=0.7835).

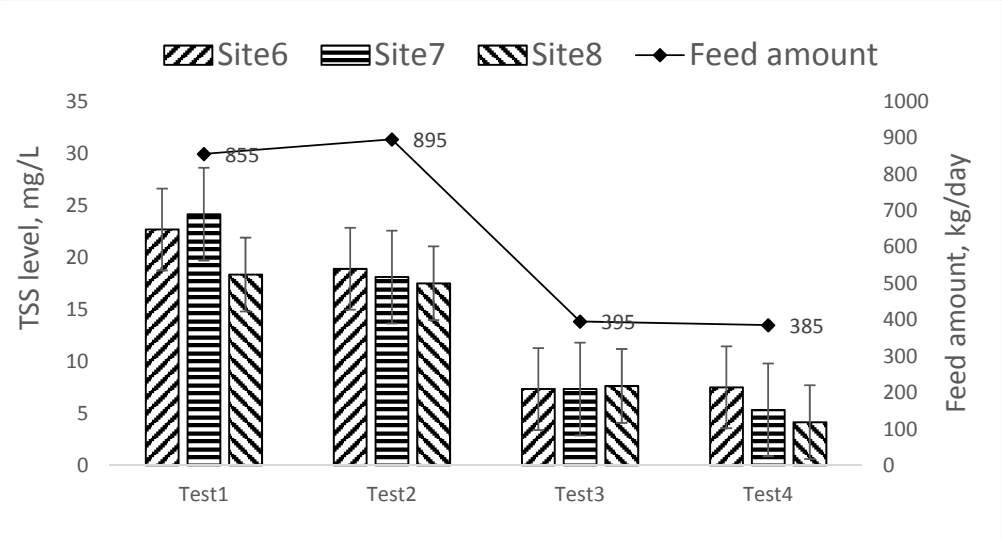

Figure 5.4.1 Variation of TSS concentration (mg/L) at different sites during four tests (Site 6: after MBBR; site 7: before drum filter; site 8: after drum filter).

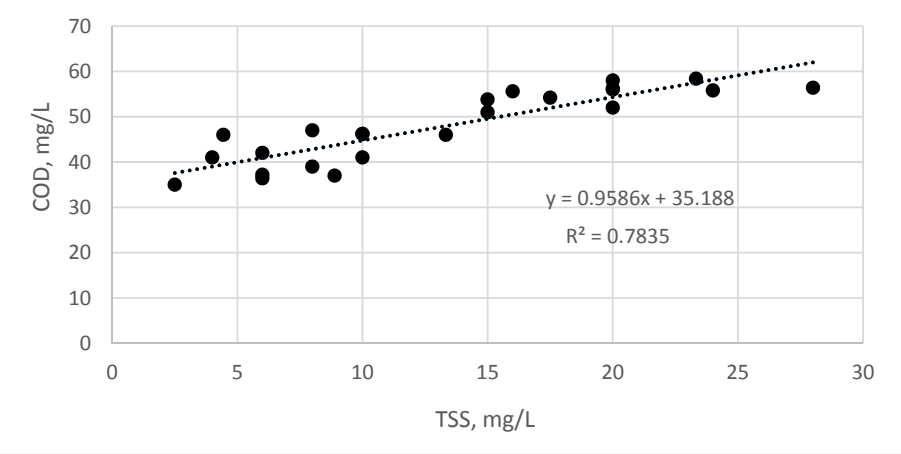

Figure 5.4.2 Relationship between TSS (mg/L) and COD (mg/L).

5.5 Turbidity and its relationship with TSS

Similar to TSS concentration variation, turbidity showed a declining trend from test 1 to 4 as well (Figure 5.5.1). Moreover, turbidity also showed declining trend between each site in one test. Turbidity declined significantly between site 3 and 8 in all tests ($P<0.05$).

As shown in Figure 5.5.2, there was strong positive correlation between TSS concentration and turbidity in a log-linear model ($R^2 = 0.917$), with a regression equation of TSS = 15.46 ln (NTU) -8.4207. The result suggested that turbidity is a suitable monitoring parameter as proxy for TSS in this case study.

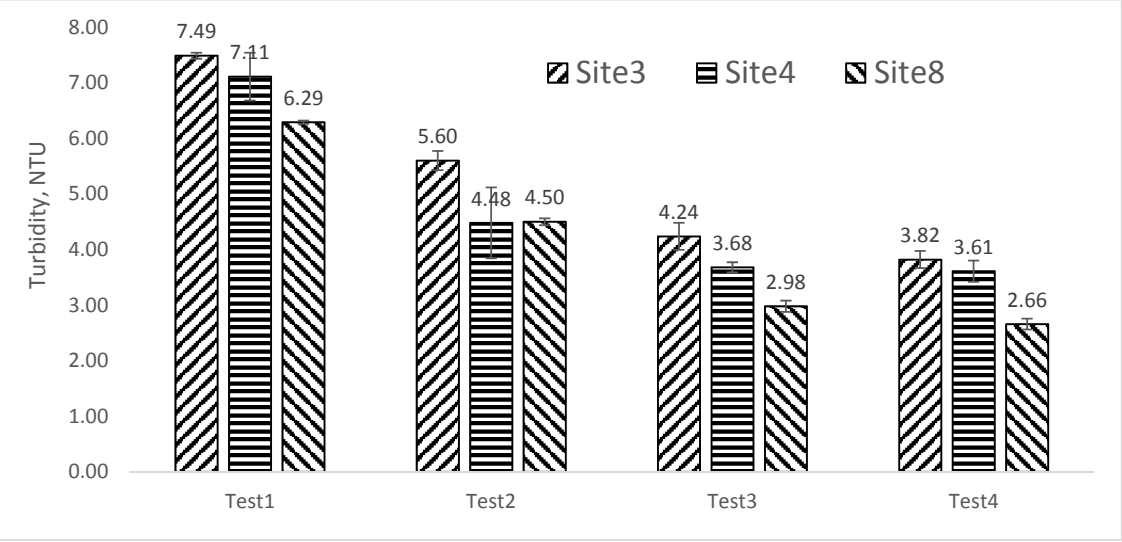

Figure 5.5.1 Turbidity (NTU) variation at different sites during four tests (n=3. Site3: before CO_2 stripper; site4: after ozone cabin; site8: after drum filter).

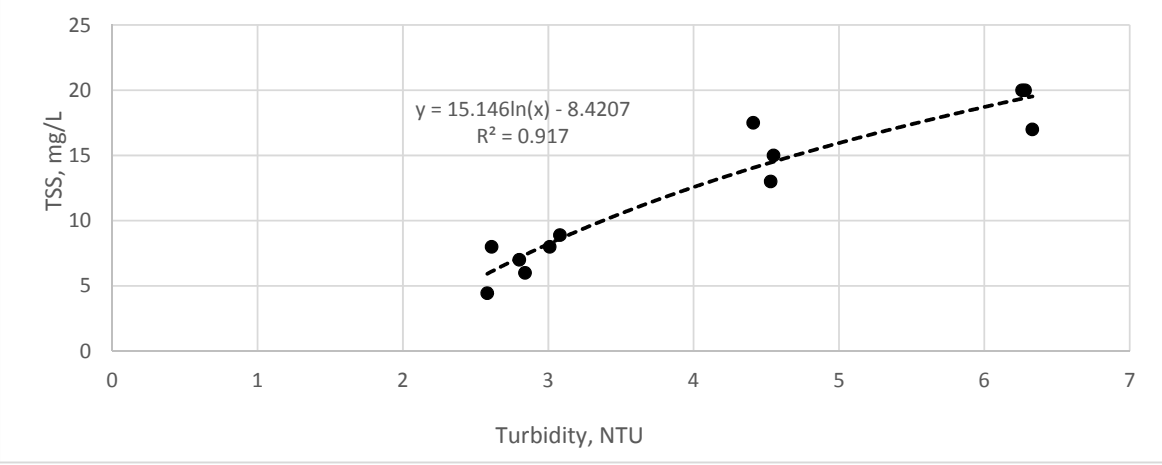

Figure 5.5.2 Relationship between turbidity (NTU) and TSS (mg/L).

5.6 Heterotrophic bacteria count in make-up water and reused water

As shown in Table 5.6, make-up water had a relatively low heterotrophic bacteria count. In addition, not even a 1-Log10 (90%) reduction was achieved in the study. The highest reduction rate was 83% in test 1 of treatment in make-up water.

Table 5.6 Heterotrophic bacteria count (CFU/mL) and reduction percent at different sites during four tests (M±SD. Site1: before ozone tank; site2: after UV; site3: before CO_2 stripper; site4: after ozone cabin).

	Make-up water							Reused water						
n=3	Site1			Site2			Reduction	Site3			Site4			Reduction
Test1	60.0	±	35.6	10.0	±	8.2	83.33%	1703.3	±	295.8	1490.0	±	283.3	12.52%
Test2	10.7	±	5.2	14.0	±	5.1	a	3233.3	±	1975.4	1333.3	±	1007.7	58.76%
Test3	33.3	±	17.5	19.3	±	1.9	42.00%	2166.7	±	1087.3	3633.3	±	793.0	a
Test4	4.7	±	2.5	2.0	±	0.0	57.14%	933.3	±	713.4	366.7	±	449.7	60.71%

a: increased heterotrophic bacteria count

There was no significant decline in heterotrophic bacteria count in make-up water (Figure 5.6.1). However, make-up water had a relatively low heterotrophic bacteria count after UV and ozone treatment, which ranged from 2.0±0.0 to 19.3±1.9 CFU/mL. In addition, turbidity declined significantly after treatment in test 3 and 4 ($P<0.05$).

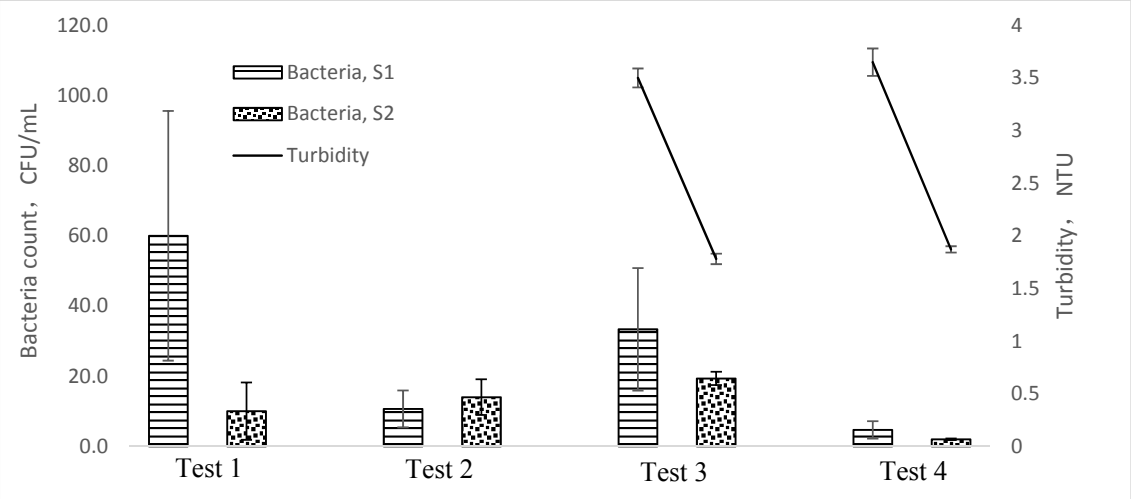

Figure 5.6.1 Variation of heterotrophic bacteria count (CFU/mL) in make-up water (Site1: before ozone tank; site2: after UV).

Similar to make-up water, there was no significant decline in heterotrophic bacteria count in reused water (Figure 5.6.2), and the heterotrophic bacteria count ranged from 366.7±449.7 to 3633.3±793.0 CFU/mL after ozonation treatment, higher than the value in make-up water. In addition, turbidity showed decline on average value after ozonation treatment.

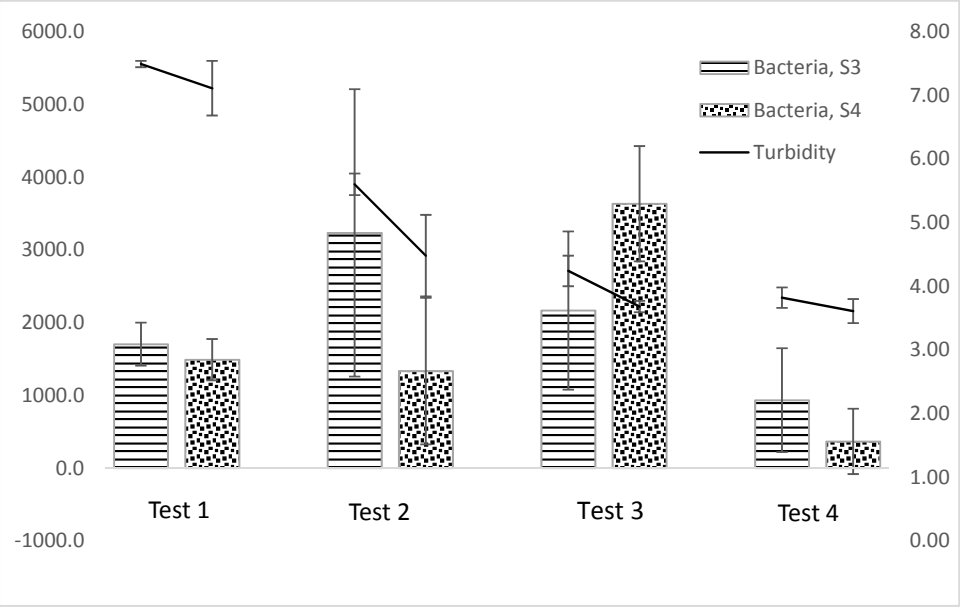

Figure 5.6.2 Variation of heterotrophic bacteria count (CFU/mL) in reused water (Site3: before CO_2 stripper; site4: after ozone cabin).

6. DISCUSSION

6.1 The experimental setup

Many water quality parameters were measured in this study, but parameters like mortality, specific growth rate (SGR) and feed conversion ratio (FCR) were not included. This was one of the drawbacks in experimental design. Because mortality and fish growth was influenced by water quality, so they can indicate whether water quality was suitable for fish growth.

From site 3 to 4, reused water passed through CO_2 stripper and flowed into the closed ozone cabin. Actually, TAN concentration was affected by both of them. However, it was impossible to take water sample after CO_2 stripper due to integrated design with the closed ozone cabin. Future investigation can be made on CO_2 strippers located outside to evaluate impacts of CO_2 stripper on TAN concentration.

Bacteria measurement was carried out in an empty room instead of sterile room, the results may be disturbed by bacteria from the atmosphere. Compact Dry AQ kits were used to detect and enumerate heterotrophic bacteria, but manufacturer did not provide technical information on the composition of plates. Enumeration were based on red spots on plates, which were caused by bacteria capable of fermentation. Since bacteria incapable of fermentation are not colored, so this may result in underestimation of heterotrophic bacteria count. Furthermore, samples were incubated at 36±2℃ rather than 22±2℃, which was closer to water temperature in the system. Therefore, this may result in an incredible heterotrophic bacteria count. In addition, yellow colonies appeared on plates disturbed counting (Figure 6.1.1), and some plates were difficult to count due to ambiguous colonies (Figure 6.1.2), which was caused by improper dilution as explained by the supplier.

Errors in TSS measurement mainly came from three aspects. First, TSS was the last parameter to measure (2-3 hours after sampling), decomposition may occur before filtration process, which may result in underestimation. Second, water at site 8 (after filtered by 25μm-mesh drum filter) contains low TSS level and 1L water (taken by two times) are needed to finish the

filtration process, while sample water taken at different time may has different TSS concentration. Third, after filtration, TSS samples were treated by sticking with another unused glass fiber and kept frozen. Weighing was done at the laboratory at NMBU rather than on site.

In turbidity measurement, due to visible suspended solids in water sample, the display on Nephelometer fluctuated in a small range and took long time to get stable display. Sedimentation may happen during this period, which could lead to underestimation.

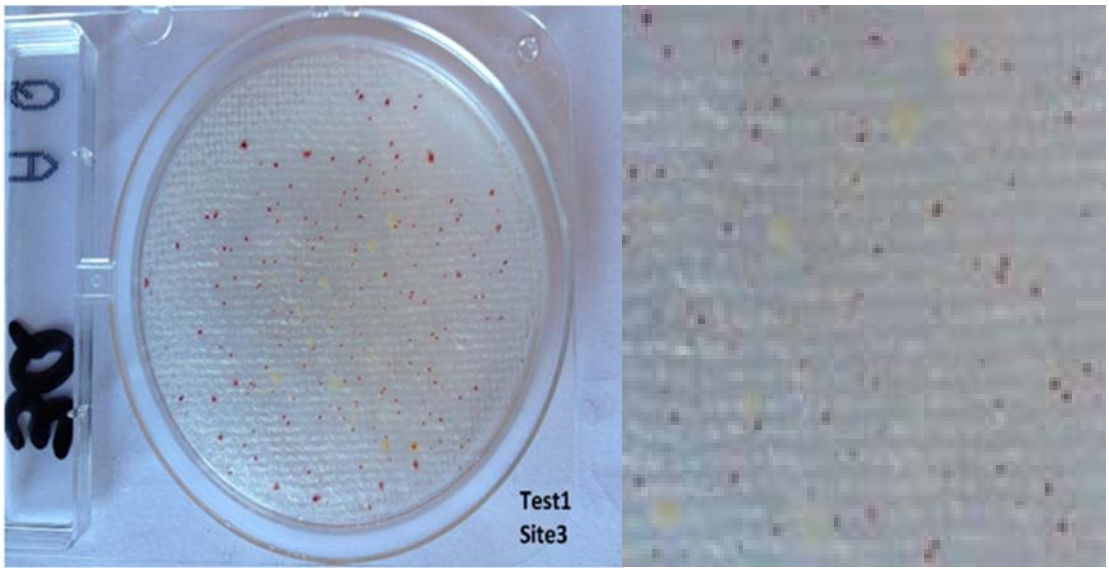

Figure 6.1.1 Yellow colonies appeared on plates disturb counting

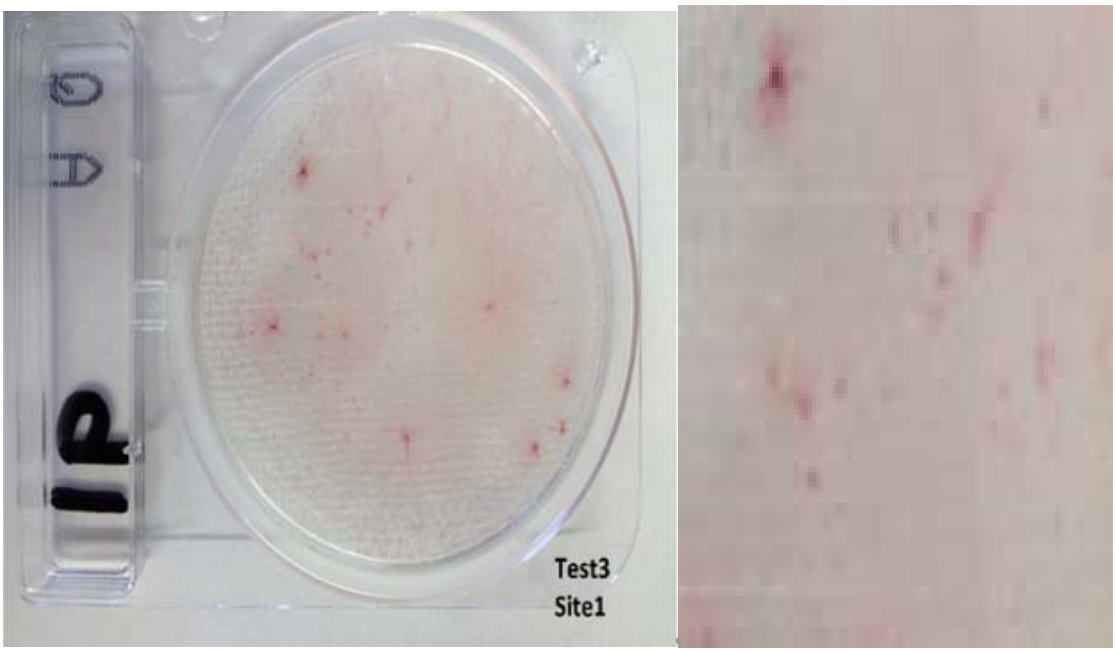

Figure 6.1.2 Ambiguous colonies disturb counting

6.2 Discussion of water quality and MBBR performance

6.2.1 TAN, NO_2-N concentration and removal rate

Due to its high toxicity to fish, free ammonia accumulation is one of the most notorious problems in RAS (Hargreaves, 1998). Molleda *et al.* (2007) study in a RAS for arctic charr (*Salvelinus alpinus*) farm found that free ammonia concentration ranges from 0.001-0.014 mg/L in outlet water from fish tanks and 0.002-0.018 mg/L in outlet water from the bio filter unit. In this study, higher values were observed. Especially in test 2, free ammonia concentration at all sites were above 0.05 mg/L, which was two times higher than the recommended maximum concentration (0.025mg/L) in intensive fish farming (Chen *et al.*, 2006). While in the other tests, free ammonia concentration at site 7 were only around 0.005 mg/L, well below the recommended value.

In the same study from Molleda *et al.* (2007), TAN concentration was around 0.251-1.520 mg/L in outlet from fish tanks and 0.246-1.577 mg/L in outlet from the bio filter unit. Higher values were observed in this study also. In test 2, TAN level was above 15 mg/L at all sites, which was higher than the recommended concentration (1.0 mg/L) for long-term exposure (Losordo *et al.*, 1998). TAN levels observed in test1 were also higher than in test 3 (0.49±0.02 to 1.44±0.23 mg/L) and test 4 (0.58±0.12 to 2.31±0.02 mg/L).

This difference can be explained by variations in feeding amount. 855 kg/day and 895 kg/day feed were used in test 1 and 2, while in test 3 and 4, it was only 395 kg/day and 385 kg/day. In addition, one day before test 2, there were uneaten feed floating in tank 15 and tank 16, and the film of first drum filter was partially blocked by uneaten feed.

In another study at a running warm water RAS for Nile tilapia, Ding (2012) found areal TAN removal rate range from 0.14-0.19 g/m^2.d. Higher efficiency was observed in this study, which ranged from 0.216 to 0.513 g/m^2.d. In test 2, when the highest TAN level (16.32±0.17 mg/L) and lowest TAN/COD ratio (3.92±0.03) was observed, the highest areal TAN removal rate

(0.513±0.186 g/m^2.d) was achieved. Similar ATR rate was found in a freshwater application of MBBR for production of brown trout and arctic char juveniles (Rusten *et al.*, 2006), where maximum ATR rate of 0.30 g/m^2.d was reached at a TAN load of 0.45 mg/L at temperature of 9 ℃.

In the same study from Molleda *et al.* (2007), NO_2-N concentration never exceed 1.1 mg/L. Higher NO_2-N concentration was observed in test 1 and 2, which ranged from 1.03 to 1.80 mg/L. While in test3 and 4, NO_2-N concentration was low and ranged from 0.35 to 0.75 mg/L. Nevertheless, these values were well lower than the recommended value (10 mg/L) for long-term exposure (Masser *et al.*, 1999). Regarding areal nitrite removal rate, it varied between 0.24 and 0.54 g/m^2.d in this study. Similar to Ding's result (2012) in a RAS for Nile tilapia, where the areal nitrite removal rate ranged from 0.19-0.29 g/m^2.d.

Regarding ATR rate, chamber 1 (varied from 0.142±0.034 to 0.439±0.329 g/m^2.d) showed higher efficiency than chamber 2 (varied from 0.074±0.143 to 0.193±0.014 g/m^2.d). Ozone residues in water might contribute to the high efficiency in chamber 1, because TAN can be primarily oxidized by ozone to other nitrogen compounds such as nitrogen gas in aquaculture systems (Schroeder *et al.*, 2011).

Concerning the percent TAN removal, except in test 2, more TAN was removed in chamber 2 (41.62±1.81% to 59.58±3.71%) than in chamber 1(10.30±1.12 % to 30.53±7.45%). This was because chamber 2 had a larger surface area than chamber 1 (58571 m^2 compared with 17677 m^2), and water had two-times longer retention time in chamber 2.

6.2.2 NO_3-N variation and feed loading rate

Feed loading rate was a crucial parameter in RAS design and management. Ding (2012) reported stable NO_3–N concentration in a RAS for tilapia ranged from 22.6 to 25.7 mg/L at a low feed loading rate (0.43-1.11 kg feed / m^3 make- up water). Higher NO_3–N concentration were observed in this study, which was in the range of 23.07±1.24 to 53.60±10.37 mg/L. The difference was caused by higher feeding load in this study (1.70-38.19 kg feed / m^3 make- up water). However, when feed loading rate ranged from 1.6-6.3 kg feed / m^3 make- up water in a RAS rainbow trout, Pedersen *et al.* (2012) reported NO_3–N concentration ranged from 54±7 to 196±10 mg/L. In this study, there was no accumulation of NO_3-N even at a high feed loading rate (38.19 kg feed/m^3 make- up water). The result indicated that MBBR in the farm function effectively at a high feed loading rate.

As the end product of nitrification, nitrate is less toxic than free ammonia and nitrite. With a 96-h LC values usually over 1000 mg/L (Timmons *et al.*, 2002). In practice, NO_3–N level will not reach such a high value, due to denitrification or daily water exchange. Actually， denitrifying activity may take place in deeper layer of biofilms, where oxygen level was low due to consumption by nitrifying bacteria (Hamlin *et al.*, 2008).

6.2.3 COD variation and COD/TAN ratio

In a study done at a trout farm (Schulz *et al.*, 2003), reported COD level of outlet water from fish tank fluctuated around 41.01 mg/L, similar results were observed in test 3 (ranged from 36.73±0.25 to 39.53±0.74 mg/L). While higher COD level were observed in other tests, which ranged from 45.40±0.28 to 66.67±0.90 mg/L.

The chemical oxygen demand to nitrogen ratio (COD/N) is a critical parameter for bio filter system design and nitrification process, because it influenced the competition between autotrophic and heterotrophic bacteria on bio films directly (Bovendeur *et al.*, 1990). In aquaculture systems, bio filter systems were supposed to operate at a low TAN concentration

in order to avoid toxic exposure. However, organic matter concentration is relatively high when compared with TAN in aquaculture systems (Zhu and Chen, 2001).

In this study, test 2 showed a low and stable COD/TAN ratio (ranged from 3.92±0.03 to 4.09±0.02). While in other tests, COD/TAN ratio surged from site 3 to 6. Accordingly, test 2 showed highest areal TAN removal rate (0.513±0.186 g/m^2.d). Ling and Chen (2005) found nitrification rate decreased exponentially with the addition of organic carbon in a laboratory biofilters. Nitrification rates of the biofilters reduced about 60–70% for a substrate concentration of 10 mg/L TAN when the COD/N ratio increased from 0 to 3 (Bovendeur *et al.*, 1990). These results indicated that organic matter removal could improve nitrification rate in a recirculating systems, because at a high COD/TAN ratio nitrifying bacteria were inhibited by heterotrophic bacteria.

6.2.4 TSS variation

TSS affect fish directly by clogging and being abrasive to fish gills (Cordone and Kelley, 1961), or stressing the fish and destroying their immune system which will result in increased disease susceptibility and osmotic dysfunction (Redding *et al.*, 1987). Bilotta and Brazier (2008) reported 6% mortality in arctic grayling fry when they were exposed to 25 mg/L TSS for 24h. The recommended TSS concentration in arctic charr culture was 15 mg/L, while in Atlantic salmon it was 20 mg/L (Molleda *et al.*, 2007).

In test 1 and 2, TSS concentration fluctuated around the recommended value, which ranged from 17.5±2.50 to 24.13±3.61 mg/L. While in test 3 and 4, lower TSS concentration was observed (below 8 mg/L). The results were similar to Twarowska (1997) founding in in a RAS for fingerling tilapia, where TSS concentration was usually less than 7.5mg/L.

The high TSS concentration in test 1 and 2 were mainly caused by overfeeding. In the first two tests 855 kg and 895 kg feed were used daily, while in test 3 and 4 it was only 395 kg and 385 kg feed were given. Especially in test 2, there was uneaten feed floating in tanks one day before sampling and drum filter was partially blocked by suspended solids.

6.3 Function of the closed ozone cabin

Ozone works effectively in fish pathogens inactivation, organic wastes removal (including color and smell removal) and nitrite oxidization (Wedemeyer, 1996). Besides, ozonation of water in recirculating systems improves fish welfare by reducing fish disease and environmental stress (Brazil, 1996).

Summerfelt *et al.* (2001) suggested that most pathogen organisms can be inactivated at ozone dosage 0.5-5.0 min*mg/L. However, the result showed no significant decline in heterotrophic bacteria count in reused water with ozone dosage of 3.34-4.56 min*mg/L, and not even a 1-Log10 reduction was achieved. The result indicated that the closed ozone cabin does not have any function in pathogens inactivation. The poor disinfection efficiency may be caused by existence of suspended solids, because suspended solids can harbor bacteria from the oxidation (Qualls *et al.*, 1983) and RAS provided a selection process that favors bacteria embed within particulate matters (Sharrer and Summerfelt, 2007).

In addition, ozone can react almost instantaneously with nitrite to nitrate, with a rate constant of 3.7×10^5 M/s (Schroeder *et al.*, 2011). This was supported by the significant decline in NO_2-N concentration between site 3 and 4 in this study ($p<0.05$). However, between site 3 and 4 water passed through both CO_2 stripper and closed ozone cabin, so it was difficult to determine which process had the main impact. Because CO_2 stripper was filled up with bio-blocks had specific surface area 80 m^2/m^3, nitrifying bacteria might establish colonies on surface and nitrification take places when water passed through it. The impact of CO_2 stripper on nitrification needs to be further investigated. In addition, both COD and turbidity showed declines on average value when water passed CO_2 stripper and the closed ozone cabin.

Therefore, when water passed through CO_2 stripper and ozone cabin, NO_2-N was first oxidized, then COD and turbidity reduction together with pathogens inactivation.

6.4 Heterotrophic bacteria count and disinfection efficiency

Sharrer *et al.* (2005) reported make-up water in a RAS for rainbow trout contained on average 1940±220 CFU /mL heterotrophic bacteria count. In this study, make-up water from the nearby lake had a relatively low heterotrophic bacteria count, which was below 60.0±35.6 CFU/mL. Sharrer and Summerfelt (2007) reported that combining ozone dosages of only 0.1-0.2 min*mg/L with UV irradiation dosages of about 50 mJ/cm^2 would reduce bacteria counts to almost zero (0–4 CFU/mL). Though higher UV (77 mJ/cm^2) and ozone (7.56 min*mg/L) dosages were used in make-up water treatment, only test4 showed similar result (2 CFU/mL). However, heterotrophic bacteria count in make-up water of the other tests after ozone and UV treatment were also low, which were below 19.3±1.9 CFU/mL.

Reused water had a low heterotrophic bacteria count (3233.3±1975.4 CFU/mL) compared with Sharrer *et al* .(2005) result in a RAS for rainbow trout, which was 21,360±4500 CFU/mL on average before treatment. Summerfelt *et al.* (2001) suggested that many pathogen organisms can be inactivated at ozone dosage 0.5-5.0 min*mg/L. In this study, the ozone dosage was 3.34-4.56 min*mg/L and heterotrophic bacteria count in reused water after treatment ranged from 366.7±499.7 to 3633.3±793.0 CFU/mL. Similar results were observed by Bullock *et al.* (1997) at a rainbow trout farm, where heterotrophic bacteria count ranged from 10^3 to 10^4 CFU/ mL, with adding 36-39 g O_3/kg feed (13-20 g O_3/kg feed in this study).

In this study, the heterotrophic bacteria removal efficiency did not reach 1-LOG10 reduction. Bullock *et al.* (1997) suggested that to disinfect thoroughly it needs much greater ozone dosages than it is typically required for simply water quality control, and adding about 25 g ozone/ kg feed (13-20 g O_3/kg feed in this study) was sufficient for both fish health and water quality improvement purposes, though this dosage would not produce even a 1-LOG10 reduction in heterotrophic bacteria count.

In addition, it is worth noting that Log10 reduction only tells how much bacteria is reduced from the start point, it tells nothing about how much is left in the water.

6.5 Turbidity as a proxy for total suspended solids (TSS)

The measurement of TSS is time consuming. As one of the least expensive and easiest parameters to measure, turbidity has been used widely to correlate as secondary parameters to TSS in many water environments (Gippel, 1989). However, most of these searches were conducted in lake or river water, little research has been done in aquaculture water.

Generally, the relationship between turbidity and TSS depends on the size, density, shape and type of the suspended solids in water, as well as on watercolor. Simple linear relationships are mostly reported, and roughly 1 NTU corresponds to 1–2 mg /L suspended solids (Rugner *et al.*, 2013). In an earlier study in urbanizing streams (Packman *et al.*, 1999), the author found strong positive correlation between TSS and turbidity (R^2= 0.96) by applying a log-linear model, with a regression equation of ln (TSS) = 1.32 ln (NTU) + C, with C not significantly different than 0 for 8 of the 9 sampled streams.

The Log-linear model in this study（TSS = 15.46 ln (NTU) -8.4207）indicated good correlation (R^2=0.917) between TSS and turbidity (Figure 5.6.1). The result strongly support the proposal as using turbidity as an easy-to-monitor proxy for concentration of total suspended solids.

6.6 Future studies

1. To study the impact of CO_2 stripper on nitrification process. Because CO_2 strippers were filled up with bio-blocks which had specific surface area 80 m^2/m^3, thus nitrifying bacteria could establish their colonies on the surface of bio-blocks. Therefore, nitrification could also take place when reused water pass through the CO_2 stripper.

2. To evaluate the feasibility of using turbidity as an alternative parameter for TSS in aquatic environment. If reliable model could be developed, it can provide convenience in water quality management. Because TSS measurement is time-consuming, while turbidity measurement is easier, faster and less expensive in comparison with TSS measurement.

7. CONCLUSION

In general, water quality at Vik was well maintained within the range for the optimal growth for salmon and rainbow trout, except in test 2 when high TAN values (ranged from 14.68±0.26 to 16.32±0.17 mg/L) was observed due to overfeeding.

Make-up water had stable quality, with temperature around 9.20-10.8℃, pH varied from 5.67 to 6.13, and a low alkalinity(around 5 mg/L as $CaCO_3$), so it was necessary to monitor alkalinity at a high water exchange rate.

MBBR functioned effectively in nitrogenous waste removal. COD/TAN ratio was low and stable in test 2 (ranged from 3.92±0.03 to 4.09±0.02). While in other tests, COD/TAN ratio surged from site 3 to 6, especially between site 5 and 6. The highest areal TAN removal rate (0.513±0.186 $g/m^2.d$) was achieved in test 2.

In general, chamber 1 had higher efficiency in areal TAN, NO_2-N and COD removal rate than chamber 2. However when regarding percent TAN reduction, more TAN was removed in chamber 2 (41.62±1.81% to 59.58±3.71%) than in chamber 1(10.30±1.12 % to 30.53±7.45%), except in test 2. This was because chamber 2 had lager surface area than chamber 1 (58571 m^2 compared with 17677 m^2), and water had two-times longer retention time in chamber 2.

Make-up water had low heterotrophic bacteria count, which ranged from 4.7±2.5 to 60.0±35.6 CFU/mL before treatment. However, not even a 1-Log10 (90%) reduction was achieved in make-up water after ozone and UV treatment. In reused water, the result showed no significant decline in the heterotrophic bacteria count, the value ranged from 366.7±499.7 to 3633.3± 793.0 CFU/mL after ozonation.

There was strong positive correlation between TSS concentration and turbidity in a Log-linear model (R^2=0.917), with a regression equation of TSS = 15.46 ln (NTU) -8.4207. The result suggested that turbidity could be used as a proxy for TSS in this study.

8. REFERENCES

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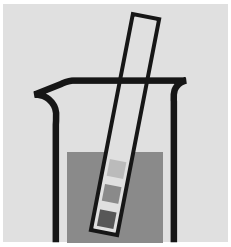
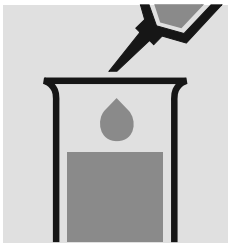
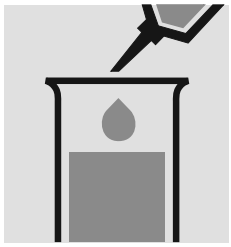
Measuring	0.05 – 3.00 mg/l NH_4-N	0.06 – 3.86 mg/l NH_4	10-mm cell
range:	0.03 – 1.50 mg/l NH_4-N	0.04 – 1.93 mg/l NH_4	20-mm cell
	0.010 – 0.500 mg/l NH_4-N	0.013 – 0.644 mg/l NH_4	50-mm cell
	Expression of results also possible in mmol/l.		



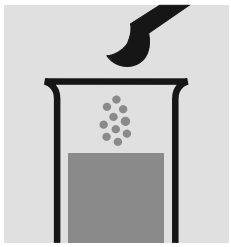
Check the pH of the sample, specified range: pH 4 – 13.
If required, add dilute sodium hydroxide solution or sulfuric acid drop by drop to adjust the pH.



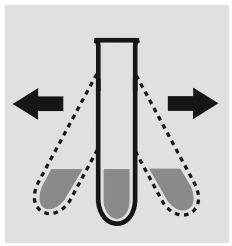
Pipette 5.0 ml of the sample into a test tube.



Add 0.60 ml of **NH_4-1** with pipette and mix.

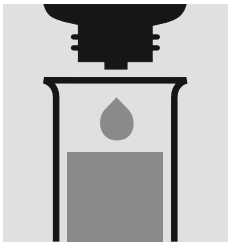


Add 1 level blue microspoon of **NH_4-2**.

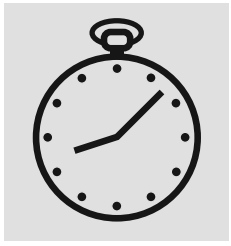


Shake vigorously to dissolve the solid substance.

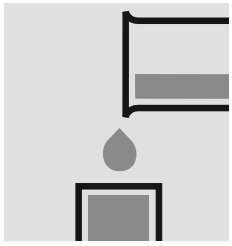
Reaction time:
5 minutes



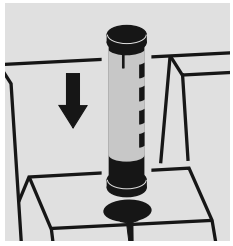
Add 4 drops of **NH_4-3** and mix.



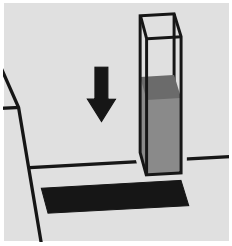
Reaction time:
5 minutes



Transfer the solution into a corresponding cell.



Select method with AutoSelector.



Place the cell into the cell compartment.

Important:

Very high ammonium concentrations in the sample produce turquoise-coloured solutions (measurement solution should be yellow-green to green) and false-low readings are yielded. In such cases the sample must be diluted (plausibility check).

To measure in the 50-mm cell, the sample volume and the volume of the reagents have to be doubled for each. Alternatively, the semi-microcell, Cat.No. 73502, can be used.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) we recommended to use Spectroquant® CombiCheck 50, Cat.No. 14695.

Ready-for-use ammonium standard solution CertiPUR®, Cat.No. 19812, concentration 1000 mg/l NH_4^+, can also be used after diluting accordingly.

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

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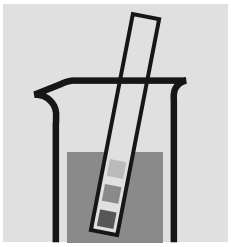
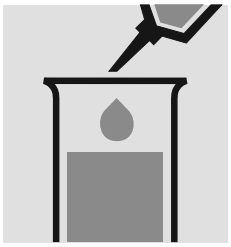
Test

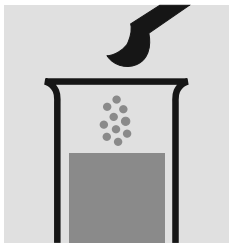
Measuring	0.02 – 1.00 mg/l NO_2-N	0.07 – 3.28 mg/l NO_2	10-mm cell
range:	0.010 – 0.500 mg/l NO_2-N	0.03 – 1.64 mg/l NO_2	20-mm cell
	0.002 – 0.200 mg/l NO_2-N	0.007 – 0.657 mg/l NO_2	50-mm cell
	Expression of results also possible in mmol/l.		



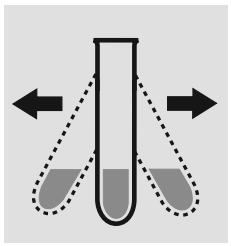
Check the pH of the sample, specified range: pH 2 – 10.
If required, add dilute sulfuric acid drop by drop to adjust the pH.



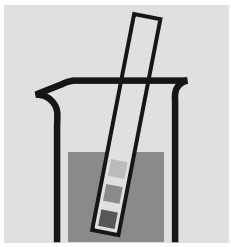
Pipette 5.0 ml of the sample into a test tube.



Add 1 level blue micro-spoon of **NO_2-1**.

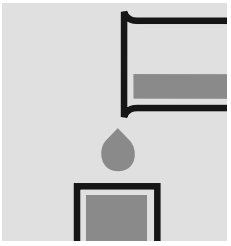


Shake vigorously to dissolve the solid substance.

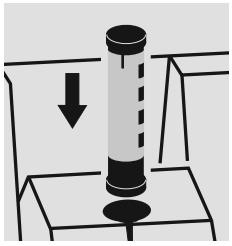


Check the pH, specified range: pH 2.0 – 2.5.
If required, add dilute sodium hydroxide solution or sulfuric acid drop by drop to adjust the pH.

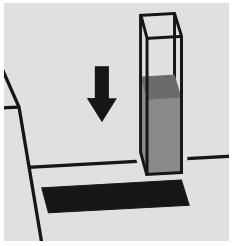
Reaction time:
10 minutes



Transfer the solution into a corresponding cell.



Select method with AutoSelector.



Place the cell into the cell compartment.

Important:

To measure in the 50-mm cell, the sample volume and the volume of the reagents have to be doubled for each. Alternatively, the semi-microcell, Cat.No. 73502, can be used.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) ready-for-use nitrite standard solution CertiPUR®, Cat.No. 19899, concentration 1000 mg/l NO_2^-, can be used after diluting accordingly.

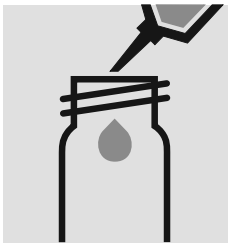
Spectroquant®

Nitrate

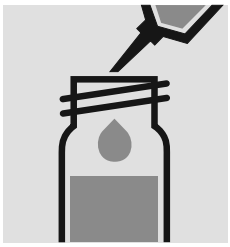
09713

Test

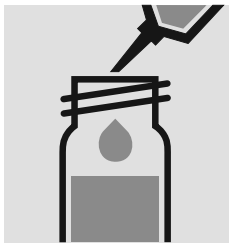
Measuring	1.0 – 25.0 mg/l NO_3-N	4.4 – 110.7 mg/l NO_3	10-mm cell
range:	0.5 – 12.5 mg/l NO_3-N	2.2 – 55.3 mg/l NO_3	20-mm cell
	0.10 – 5.00 mg/l NO_3-N	0.4 – 22.1 mg/l NO_3	50-mm cell
	Expression of results also possible in mmol/l.		



Pipette 4.0 ml of **NO_3-1** into a dry empty round cell (Empty cells, Cat. No. 14724).

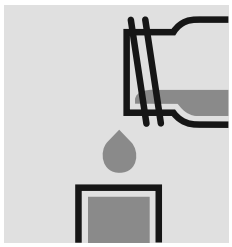


Add 0.50 ml of the sample with pipette, **do not mix.**

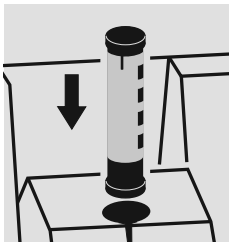


Add 0.50 ml of **NO_3-2** with pipette, close the cell with the screw cap, and mix. **Caution, cell becomes hot!**

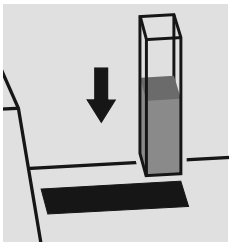
Reaction time: 10 minutes



Transfer the solution into a corresponding rectangular cell.



Select method with AutoSelector.



Place the cell into the cell compartement.

Important:

To measure in the 50-mm cell, the sample volume and the volume of the reagents have to be doubled for each. Alternatively, the semi-microcell, Cat.No. 73502, can be used.

Note:

Empty cells with screw caps, Cat.No. 14724 are recommended for the preparation. These cells can be sealed with the screw caps, thus enabling a hazard-free mixing of the sample.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) we recommended to use Spectroquant® CombiCheck 20, Cat.No. 14675.

Ready-for-use nitrate standard solution CertiPUR®, Cat.No. 19811, concentration 1000 mg/l NO_3^-, can also be used after diluting accordingly.

To check for sample-dependent effects the use of addition solutions (e.g. in CombiCheck 20) is highly recommended.

Spectroquant®

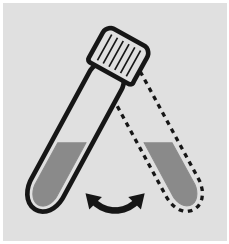
COD

Chemical oxygen demand

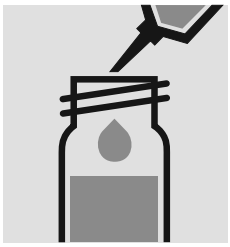
14560

Cell Test

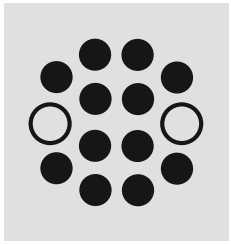
Measuring range:	4.0 – 40.0 mg/l COD or O_2 Expression of results also possible in mmol/l.



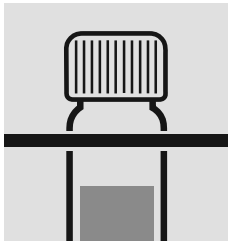
Suspend the bottom sediment in the cell by swirling.



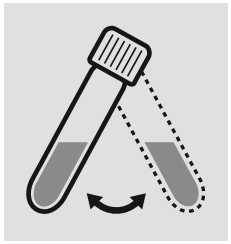
Carefully pipette 3.0 ml of the sample into a reaction cell, close tightly with the screw cap, and mix vigorously. **Caution, the cell becomes hot!**



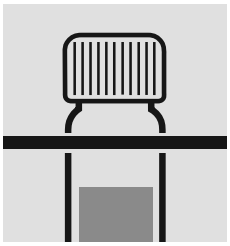
Heat the reaction cell in the thermoreactor at 148 °C for 2 hours.



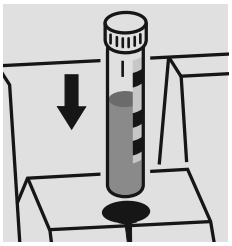
Remove the cell from the thermoreactor and place in a test-tube rack to cool.



Swirl the cell after 10 minutes.



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

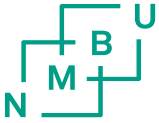


Place the cell into the cell compartment. Align the mark on the cell with that on the photometer.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) we recommended to use Spectroquant® CombiCheck 50, Cat.No. 14695.

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



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