The effects of different combinations of fixed and moving bed bioreactors on rainbow trout (*Oncorhynchus mykiss*) growth and health, water quality and nitrification in recirculating aquaculture systems

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Highlights

- Organic material accumulated in the two-moving-bed systems
- Nitrite concentrations increased in the two-fixed-bed systems
- Different bioreactor designs did not affect fish health or growth

Abstract

The effect of bioreactor design on nitrification efficiency has been well studied, but less is known about the overall impacts on water quality. Besides nitrification, submerged fixed bed bioreactors (FBBR) trap fine solid particles, whereas moving bed bioreactors (MBBR) grind solids, possibly increasing solids and particle accumulation in the system. In this experiment, the effects of different combinations of fixed bed and moving bed bioreactors on water quality, solids removal, particle size distribution, fish health based on histopathological changes and nitrification efficiency were studied in laboratory scale recirculating aquaculture systems (RAS) with rainbow trout (*Oncorhynchus mykiss*). Three set-ups with triplicate tanks were used: 1. two consecutive fixed bed bioreactors (FF); 2. a fixed bed bioreactor followed by a moving bed bioreactor (FM) and 3. two consecutive moving bed bioreactors (MM). Fish performance was not influenced by the design of the bioreactor, specific growth rate (SGR) being between 1.59 and 1.64% d^{-1} and feed conversion ratio (FCR) between 0.95 and 0.98. Water nitrite concentration was higher in the FF systems compared to FM and MM systems, whereas the average total ammonia nitrogen concentration (TAN) was not influenced by the treatments. Nitrification rate, which was measured in the laboratory, followed the water nitrite levels, indicating highest total ammonium oxidation rates in the MM systems. UV254 absorbance and total organic carbon (TOC) concentrations were higher in the groups with moving bed systems, indicating accumulation of organic substances in the circulating water. The total volume of particles was higher in the MM systems as compared to the FF systems. The total solids balance was similar in all the bioreactor groups, since the

removal of solids by the FBBR backwash was compensated by the drum filter in the FM and MM systems. In general, no significant histopathological difference in gill, kidney, heart and liver tissue were observed between the RAS treatment groups and the flow-through treatment.

Keywords: biofiltration; histopathology; particle size distribution; water quality monitoring

List of abbreviations

FBBR	Fixed bed bioreactor
FCR	Feed conversion ratio
FF	Two consecutive fixed bed bioreactors
FM	Fixed bed, followed by moving bed bioreactor
LEH	Lamellar epithelial cell hyperplasia
MBBR	Moving bed bioreactor
MM	Two consecutive moving bed bioreactors
PSD	Particle size distribution
SGR	Specific growth rate
TAN	Total ammonia nitrogen
TGC	Thermal growth coefficient
TOC	Total organic carbon
TS	Total solids

1. Introduction

Nitrifying bioreactor operation and management is one of the most important and complex steps in recirculating aquaculture systems (RAS) (Badiola et al., 2012; Svobodova et al., 2005). Typical RAS use so-called fixed-film bioreactors, where biofilm is formed on artificial plastic carrier media or media generated from natural substances such as sand and stones (Malone and Pfeiffer, 2006). Bacteria in the media convert toxic ammonia into less toxic nitrate in a two phase nitrification process. The nitrification process allows lower water usage rates, therefore decreasing the volume of effluents requiring the treatment before discharged into the environment. There is a wide variety of nitrifying bioreactors used in RAS, which all have particular strengths and weaknesses with no single reactor type being dominant (e.g. Timmons and Ebeling, 2013).

The nitrification capacity of the following bioreactor types has been widely studied: moving bed bioreactors (MBBR) (Kamstra et al., 2017), fixed bed bioreactors (FBBR) (Pedersen et al., 2015), fluidized-sand biofilters (Summerfelt, 2006), rotating biological contactor (Brazil, 2006) and trickling filters (Greiner and Timmons, 1998; Lekang and Kleppe, 2000). Besides nitrification, different bioreactor types can also have other impacts on water quality, depending on how they are designed and operated. Trickling filters, MBBRs and RBCs are constantly interacting with air, which increases the oxygen (O_2) levels and reduces carbon dioxide concentration (CO_2) (Timmons and Ebeling, 2013). However, there is very little information on how the choice of bioreactor design can affect fish health and water quality parameters.

The moving bed bioreactor was designed in Norway in the late 1980s (Rusten et al., 2006). The reactor chamber is agitated continuously with compressed air or mechanically, the carrier media being constantly moved so as to create a scrubbing effect against each other. Because of that scrubbing effect, the reactor shears solid particles, leading to the accumulation of the total amount of particles in the system (Fernandes et al., 2017). These types of reactors are easy to operate, because there is a low head loss and no need for backwashing. In addition, the constant movement enables efficient use of the whole reactor volume, and mixing with air provides oxygen for the nitrification process. Because of scrubbing, surplus microbial biomass created in the biofilm detaches from the carrier media and is later removed from the system either by outflow or in solids removal units (Ødegaard, 2006).

Fixed bed bioreactor or fixed bed biofilm reactor (FBBR) is a reactor type, where carrier media is structurally fixed in the reactor chamber (Kadic and Heindel, 2014). Depending on the fixed media type, the reactor can be susceptible to clogging and must be backwashed frequently (Schlegel and Koeser, 2007). When using small carrier media, suspended solids particles are commonly trapped in these reactors (Fernandes et al., 2017). The distribution of flow into the reactor and inside the reactor is important: turbulent flow can cause uneven distribution of substrate in the reactor and the total effective surface area for nitrification may be diminished. Turbulent flow might also create pockets, where oxygen can be depleted and hydrogen sulphide might form.

Since O_2 is added and CO_2 is removed mainly in the other compartments of RAS, the main water quality difference between FBBR and MBBR is probably the fate of solid particles in the reactor. High suspended solids loads have been reported to cause sub-lethal stress and damages to gill structure in some fish species (Au et al., 2004; Bilotta and Brazier, 2008). Thus, the amount of solid particles may influence fish health and welfare. In addition, there is a positive correlation between bacterial numbers and the surface area of particles (Pedersen et al., 2017), which may indicate that MBBR accumulates more bacteria in the circulating water than FBBR.

In this study, we compared two widely used bioreactor types: moving bed and fixed bed bioreactors. The comprehensive approach was used for comparing the effects of different bioreactor setups on ammonium removal rates, fish health in terms of histopathological lesions and growth parameters, water quality, solids accumulation and microbial dynamics. Our hypothesis was that the accumulation of solids in the circulating water causes histopathological changes and chronic stress in the fish, which affect fish growth and feed efficiency.

2. Materials and methods

2.1. Experimental setup

The experiment was carried out in the Natural Resources Institute Finland (Luke) Laukaa fish farm using an experimental RAS platform. The platform has 10 individual freshwater recirculating systems, each consisting of a 500 l bottom drained plastic rearing tank (Arvo-Tec, Joroinen, Finland), feed collector unit, 24 cm swirl separator (Eco-Trap Collector1, Pentair Aquatic Eco-Systems, Minneapolis, USA), drum filter with 60 µm filter panels (Hydrotech HDF501, Veolia, Paris, France), 2 separate 147 l bioreactor tanks (Arvo-Tec, Joroinen, Finland), trickling filter acting as a forced-ventilated cascade aeration column

(Bio-Blok® 200, EXPO-NET Danmark A/S, Hjørring, Denmark) and pump sump (Fig. 1). Water pH was adjusted to 7.2 in pump sump with diluted sodium hydroxide using automated system (Prominent, Heidelberg, Germany). Sodium bicarbonate was dosed to the inlet water source to achieve an alkalinity of 50 mg l^{-1} ($CaCO_3$) in the RAS replacement water. Oxygen saturation was kept above 80% in the fish tanks. The system is described in more detail by Pulkkinen et al. (2018).

In the trial, three bioreactor setups were compared with triplicate units: Treatment 1. two consecutive fixed bed bioreactors (FF); Treatment 2. fixed bed bioreactor followed by moving bed bioreactor (FM) and Treatment 3. two consecutive moving bed bioreactors (MM). Two bioreactors per RAS unit were used, so that all units had similar amount of bioreactors. The experiment lasted 14 weeks. In one treatment group (FF), only two units existed for the second half of the experiment due to a technical failure with pH in one tank in week 8 of the experiment. A separate 500 l flow-through tank was used to grow fish of the same origin with same feed, serving as a flow-through treatment for fish histopathological sampling. Water temperature was adjusted to 16 °C by controlling the air temperature and in the flow-through group by controlling the inlet water temperature.

Similar plastic (PP) carrier media (RK Biolements heavy in fixed bed systems and medium in moving bed systems, RK Plast A/S, Skive, Denmark), tank hydraulic retention time and make-up water flow were used and measured constantly in all RAS units (Table 1). Carrier media, used in two earlier experiments, was mixed four weeks before the trial started, and divided evenly between the bioreactors to ensure similar bacterial seed in all the RAS units. In FF and FM units, the first bioreactor was backwashed once every two weeks. In FF units the second bioreactor was backwashed once every four weeks. The FBBR backwash water amount was not taken into account in the make-up water flow calculations, because it increased the total water volume by less than 4%.

2.2. Fish and feeding

Three weeks before the trial started, a total of 820 one year old rainbow trout (*Oncorhynchus mykiss*) (average weight 99 g) originating from the National JALO-selective breeding programme (Natural Resources Institute Finland, Tervo, Finland) were divided into the 9 RAS units. When the trial started, the fish were weighed and their biomasses were equalized. The fish were weighed twice during the experiment at weeks four and eight and group weighing was used in all of the weightings. Fish were fasted one day prior to and after the weighing. Feeding was carried out with a commercial feeding system (T Drum 2000, Arvo-Tec, Joroinen, Finland) 10–14 times per day. Feed intake rate was constantly monitored using sieve in the tank outlet and uneaten feed pellets were calculated. Feed company feeding table was used for feeding rate and it was reduced by 0.1 %-unit, when uneaten feed was observed. 1:1 mixture of two commercial diets was used to ensure that the results can be better generalized across various commercial feeds. Diets were produced by Raisioaqua (Circuit Red 5 mm, Raisio, Finland) and BioMar (Orbit 929 4.5 mm, Aarhus, Denmark). The crude protein and lipid contents of the diets were 43% and 42%, and 26% and 31%, respectively.

The feed conversion ratio (FCR) was calculated as: FCR = F / G, where F = cumulative feed intake between weightings and G = total tank biomass gain between weightings. Specific growth rate (SGR) was

calculated as: SGR = $(\ln(W_{i+1}) - \ln(W_i)) / (t_{i+1} - t_i) \times 100$, where W = average fish weight at given time and $t_{i+1} - t_i$ = duration of feeding days. The thermal growth coefficient (TGC) was calculated for the whole experiment according to Jobling (2003) as: TGC = $((W_e^{1/3}) - (W_i^{1/3})) \times (T \times t) \times 1000$, where W_e = average fish weight in the end, W_i = average fish weight at the beginning, T = average water temperature, t = duration of feeding days.

2.3. Histopathological sampling and analysis

Tissue samples (gill, kidney and liver) from 5 fish per tank were collected at the start of the experiment, twice during the experiment and again at the end of the experiment, at approximately one month intervals. The second gill arch from the right hand side was sampled and sectioned parasagittally. Kidney tissue was sampled as approximately 2 cm long sections from the distal third of the kidney and sectioned transversely. Liver tissue was sampled in approximately 1×1 cm sections and sectioned sagittally. The tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm and stained with haematoxylin and eosin (H&E) according to standard laboratory practice. The sections were examined using light microscopy.

The histopathological changes were reported on a scale from 0–3: minimal, mild, moderate and severe as described by Wolf et al. 2015. One section per tissue and per fish was examined.

The following parameters were studied and classified according to the severity of the lesions:

Gills: Lamellar epithelial cell hyperplasia (LEH): proliferation of the squamous epithelial cells lining the gill surface. General diffuse proliferative branchitis: filling of interlamellar spaces by a mixed population of epithelial and inflammatory cells. Focal branchitis: a local unspecific inflammatory change consisting mainly of mononuclear, lymphocytic cell types involving a smaller area, usually only a few lamellae. Lamellar fusion: one or more interlamellar sulci filled by proliferating pavement cells (with or without increased mucous cells, chloride cells, and/or leucocytes). Lamellar adhesion: the often focal attachment of adjacent lamellae with little or no evidence of cell proliferation. Lamellar thrombosis: formation of blood clots inside lamellar capillaries consisting of fragmented thrombocyte nuclei and/or pink fibrinous material within the distended capillaries.

Kidney: Tubular necrosis: Necrosis of tubular epithelial cells. Renal mineralization: mineralized material intraepithelially or intraluminally. Number of melanomacrophage centres or pigmented macrophage aggregates (PMAs): centres of mainly histiocytic macrophages that contain hemosiderin, melanin, lipofuscin, and/or ceroid pigments and that serve as repositories for end-products of cell breakdown.

Liver: Hepatocellular cytoplasmic vacuolation: intracytoplasmic vacuoles containing glycogen or lipids. Hepatitis/cholangiohepatitis: infiltration of acute or chronic inflammatory cells in liver tissue or around bile ducts.

2.4. Water sampling and analysis

Total ammonia nitrogen, nitrite and nitrate were analysed once a week from the tank outlet water using a spectrophotometer (Procedure 8038 Nessler, LCK341/342 and LCK340 respectively. DS 3900, Hach,

Loveland, USA). Alkalinity was analysed once a week with a standard method of titration (ISO 9963-1:1994) (TitraLab AT1000, Hach, Loveland, USA).

Particle size distribution (PSD) was analysed from the tank water and from the water taken from top of the 2nd bioreactor at week 13 (S4031, PAMAS, Rutesheim, Germany). Optical analyses covered particle sizes from 1 µm to 200 µm. A simple comparison of PSD between treatments was made by calculating the β-values (slope of log10 frequency versus log10 particle size) according to Patterson et al. (1999). Total particle surface area and volume were calculated by using the given particle size diameter (assumed sphere) multiplied by the total number of particles.

Particle counts were also measured from tank water with a CASY cell counter with a capillary size of 45 µm (Model TT, OLS OMNI Life Science GmbH, Basel, Switzerland) at week 14. Measurement principal is based on pulse area analysis, where low voltage field is cast through the samples. Measurement range was between 0.8 µm to 30 µm. The 100 ml water samples were frozen before analysis. Triplicate measurements per water sample were analysed using a sample size of 200 µl.

Total organic carbon (TOC) and UV254 (turbidity corrected) were monitored online at 6 minute intervals in the fish tanks with a UV/VIS spectrometer (5 mm open path length, spectro::lyser, s::can, Vienna, Austria). Carbon dioxide concentrations were monitored in the fish tanks at 6 minute intervals with a carbon dioxide sensor (Franatech, Lüneburg, Germany). Two hour average values are presented for these online measurements.

2.5. Solids sampling

Sludge was collected twice during the trial at weeks 7 and 11 for solids analysis. Sludge from swirl separators was collected using a 0.31 litre tube placed at the bottom of the separators. The collection period lasted six hours. Drum filter backwash water was collected for 16 hours, then weighed and mixed, after which a subsample of 1 litre was collected. Fixed bed bioreactors were cleaned by vigorous agitation with air, and one litre samples were collected from the top of the reactor and from the outlet pipe. At week 11, water collected only from the top of the reactor was used because there was no difference between these sampling points. All solids samples were put into a container and oven dried (+ 80 °C) for two days.

Total solids (g kg^{-1}) were calculated for FBBR and drum filter as: $TS = (m_d - m_t) / S \times V / F$, where m_d = dried subsample mass (g), m_t = container mass (g), S = sample size (l), V = total outflow volume (l d^{-1}), F = Feed intake (g d^{-1}). Total solids (g kg^{-1}) were calculated for the swirl separator as: $TS = (m_d - m_t) \times 4 / F$, where m_d = dried subsample mass (g), m_t = container mass (g), F = feed intake (g d^{-1}).

2.6. Bioreactor nitrification rates

Bioreactor nitrification rates (g NO_x h^{-1}) were measured at the last week of the experiment, following principles described by Jäntti et al. (2011). For the incubations, inlet water and carrier media were collected from each bioreactor tank and transferred to the University of Jyväskylä. In the laboratory, carrier media were divided into experimental vials (n = 30 per vial) with 360 ml inlet water, where $^{15}NH_4^+$

was added (final concentration of 5 mg/L; 10—15 atm%). To ensure complete nitrification, the carrier media was incubated for 3 hours at *in situ* temperature and under constant mixing by magnetic stirring bars (150 rpm). To measure ammonium and nitrate concentrations and the stable isotope composition of nitrite and nitrate, water samples were taken at the beginning of the experiment, and after 1.5 and 3 hours. Water samples were filtered with 0.2 um syringe filters and frozen immediately. Later, nitrate, nitrite and ammonium concentrations were measured with a spectrophotometer (Lasa 100, Hach, Loveland, USA). The stable isotope composition of nitrite and nitrate was measured using the denitrifier method (Sigman et al., 2001). Briefly, 20 nmoles of sample NO_{2+3} were converted to N_2O by cultured denitrifying bacteria (*Pseudomonas chlororaphis* strain DSM 6698), which lack the enzyme responsible for N_2O reduction and the isotopic composition of N_2O was measured using the IsoPrime 100 CF-IRMS with a TraceGas preconcentrator interface.

2.7. Statistics

The effects of bioreactor design on nitrification efficiency, FCR, SGR, TGC, PSD and TS were analysed using one-way ANOVA, and Tukey's post hoc test was used for comparing the effects between treatments, which takes the uneven sample sizes in the end of the experiment into account (Rusticus and Lovato, 2014). A nonparametric Kruskall-Wallis test was used for total particle counts when assumptions were not met for the parametric test. Effects of bioreactor design on water quality parameters were analysed using Mixed ANOVA, where bioreactor design type (between subjects) and measurement week (within subjects) were factors. The Bonferroni post hoc test was used for comparing effects between treatments. For online measurements, daily average values were used. Statistical analyses were done with SPSS (IBM SPSS Statistics, Armonk, USA) wherein 95 % confidence interval was used.

3. Results

3.1. Fish growth and histopathology

No significant differences were found between treatments for FCR and SGR during the trial or TGC for the whole experiment (Table 2). For the whole experiment, average feed loads were 30.09 kg (± 0.15 kg), 30.88 kg (± 0.35 kg) and 30.39 kg (± 0.28 kg) in the FF, FM and MM groups, respectively.

The most significant histopathological changes were noted in gill tissue (Table 1, supplementary material). The severity scores for both lamellar epithelial cell hyperplasia (LEH) and focal branchitis were slightly elevated at the beginning of the trial, for focal branchitis only in the FF group, and for LEH in all groups including the flow-through system.

In kidney and liver tissue, no notable histopathological changes were seen during the experiment. The PMAs noted during the experiment were mild to moderate, and no notable differences in their occurrence over time, or differences between treatment groups or control group, were noted. The inflammatory changes noted in this experiment were also minor and did not show any increase during the course of the experiment.

3.2. Water quality

There was no difference in the TAN values between treatment groups, whereas nitrite values decreased throughout the experiment in all groups. In the FF group, nitrite values were significantly higher than in the FM and MM groups ($P < 0.01$). Nitrate values were higher in the MM group in comparison to the FF ($P < 0.01$) and FM groups ($P < 0.01$) (Fig 2.).

Total organic carbon, UV254 and CO_2 values were significantly different between the treatments ($P < 0.01$). TOC was lower in the FF group as compared to the FM and MM groups. The UV254 value was lowest in the FF group, and highest in the MM group. CO_2 concentration was highest in the FF group ($P < 0.01$), but there was no significant difference between the FM and MM groups (Fig. 3).

3.3. Total solids and PSD

The sum of total solids removed from the RAS units and solids removed from the swirl separators did not differ between the treatments (Fig. 4). Solids removal by the drum filters was significantly affected by the bioreactor systems ($P < 0.01$). In the RAS with two moving bed bioreactors, drum filters removed solids the most, whereas in the RAS with two fixed bed bioreactors, solids removal by drum filters was the lowest. In the FM group, drum filter solids removal was lower than in the MM group, but it was not statistically significant ($P = 0.051$).

In fish tanks, particle size distribution values (β-value, total amounts, surface area and volume) were not significantly different between the treatments, whereas differences were observed in water samples taken after biofiltration. The β-values in water sampled after the second bioreactor was significantly higher in the FF group compared to MM ($P < 0.05$), indicating that RAS with two fixed bed bioreactors has a larger share of particles in small sizes (Table 3). Total particle amounts and surface area in the biofiltered water were not affected by the treatments, whereas total particle volumes were significantly higher in the MM group compared to the FF group ($P < 0.05$). Over 80% of the particles were below 3 µm in the FF and FM group and over 90% in the FF group (Fig. 1, supplementary material).

Although treatments with moving bed bioreactors had higher particle counts measured with the CASY cell counter, the counts were not significantly different between the treatments due to high within-treatments variance (Kruskal-Wallis $P = 0.24$; Fig. 5).

3.4. Nitrification

The nitrification rate was significantly different between the treatments ($P < 0.01$). In the FF group, the nitrification rate was lowest, but there was no difference between the MM group and FM group ($P = 0.07$). The nitrification rate did not differ between the first and second moving bed bioreactor, whereas in the FF group, the second FBBR had a lower nitrification rate than the first FBBR ($P <0.01$) (Fig. 6).

4. Discussion

4.1. Fish performance

In general, fish grew well and there were minor mortalities. However, one tank was lost due to pH probe failure. We did not see any differences in fish growth between the different bioreactor configurations, even when some difference was seen in the water quality.

We noticed higher CO_2 and NO_2-N levels in the FF group. Good et al. (2010) reported that elevated CO_2 concentration up to 24 mg l^{-1} did not affect rainbow trout growth or health. In contrast, Kahn et al. (2018) noticed that CO_2 concentration in RAS water had a negative linear correlation with Atlantic salmon (*Salmo salar*) growth. This means that there is no threshold value for CO_2 where growth would decrease: the higher the concentration the more it affects the growth. In addition, elevated nitrite concentration can cause several physiological disturbances in aquatic animals, leading to decreased growth (Aggergaard and Jensen, 2001; Jensen, 2003) and even death (Svoboda et al., 2005).

In contrast to the FF group, we noticed a higher organic material load and NO_3-N levels in the MM group. Davidson et al. (2014) recommended 75 mg l^{-1} as the maximum level of nitrate for rainbow trout. This is the level where negative impacts on long term health were seen. In addition to nitrogen compounds, solids can also have detrimental effects on fish performance. Particle accumulation in fish gills has been shown to cause inflammatory responses (Lu et al., 2018) and stress (Au et al., 2004). However, Becke et al. (2018) studied the long term effect of a high suspended solids load in RAS and despite the high load, they did not find rainbow trout histopathology or growth indicators to be significantly affected.

Taken into account all of the above, all treatment groups had water quality parameters, which could have affected the growth negatively. This might be one reason, why differences between treatment groups were not observed in growth and health. In addition, rainbow trout can be tolerant to different water qualities, thus observed differences for water quality between treatments might not be biologically relevant or within treatment variability was too high to observe any differences.

For histopathological lesions in general, only minor differences between the different treatment groups or the flow-through system were seen. Most lesions were minimal to mild, and thus clinically nonsignificant. The only clinically significant moderate changes were noted in gill tissue as an increase in lamellar epithelial cell hyperplasia (LEH), which is a common, non-specific lesion seen in subacute to chronic gill damage, and in focal branchitis (Fig. 2, supplementary material). These changes were noted also in the flow-through system. Mild, clinically nonsignificant gill lesions were noted also at the start of the experiment (T0). These changes correlate partly with noted differences in water nitrogen compounds and UV254 measurements, however, no water parameters were measured for the flow-through system. The gills are structures with a large surface area in direct contact with water, and as such are often the first tissue to show changes when water quality is suboptimal. Gills show a remarkable regenerative capacity (Ferguson, 2006) and can adapt to less optimal water quality over time (Kolarevic et al., 2012). No changes in liver or kidney tissue were noted in this experiment. Melanomacrophage centres exist in normal kidney tissue of fish and they increase with age, however an excess or increase can be seen in chronically stressed fish. A lymphoid inflammatory reaction located around bile ducts, cholangiohepatitis, can be seen in connection with parasitic infections, but may also be connected with unspecific immune mediated reactions and may have a connection with water

quality. None of these lesions were noted during the experiment; however, a prolonged exposure time of harming substances might be needed in order to provoke some of the studied changes in these organs.

4.2. Water quality

Online spectrometric water quality monitoring can provide useful information about short period fluctuations in the water quality, which cannot be seen in the manual water sampling. However, there are lots of substances that absorb light in the same wavelengths and affect the interpretation of the results. In addition, sensitivity can be weak, and accuracy is tolerable only above certain threshold values (Carré et al., 2017). The UV254 absorbance values correlate well with dissolved organic carbon (DOM) and dissolved aromatic carbon in particular (Weishaar et al., 2003), making it a useful indicator of biological substances in the water. When one or two MBBR were used in RAS unit, an increasing UV254 value was measured. Bacterial biomass was increasing in the bioreactors during the operation and this surplus biomass was removed from the FBBR when backwashed. In MBBR, surplus biomass was constantly removed into the circulating water, which increased the amount of organic matter in the water, as was seen in the UV254 absorbance values during the trial. TOC fluctuations followed the UV254 fluctuations, but there were no differences in the TOC values between the FM and MM groups.

Particle size distribution was measured, when systems were considered to be in their steady state. In RAS, small particles typically accumulate in the system, which is seen as high β-values (Patterson et al., 1999). In the present trial, total particle volume was highest in the MM group, which followed the overall water quality values. The same amount of solids was introduced into every RAS unit via fish feed and removed by water treatment units. Solids trapped and later removed by FBBR were removed by drum filter in units with MBBR. Thus, drum filters were compensating particle accumulation in the MM group, even though mesh size was 60 µm. Total particle counts measured with the CASY cell counter were between 15 and 60-fold higher than those measured with the PAMAS optic particle counter. The most likely explanation is that the cell counter is much more accurate in small size classes and there is disintegration of possible cell aggregates in the freezing period. Michaud et al. (2006) found up to 800,000 free bacterial cells per ml in biofilter effluent, which indicates that the majority of small particles are bacterial cells. Thus, total particle counts measured with the cell counter was considered to measure total bacterial counts in the water. As expected, total bacterial counts were somewhat higher in the units with moving bed bioreactors, but the difference was not significant between the groups.

In the units where MBBR was in use, CO_2 concentrations were lowest. Mixing the MBBR with compressed air was ventilating CO_2 out of the systems, but there were no differences in concentrations if one or two MBBRs were used.

4.3. Nitrification

Continuously decreasing nitrite values during the trial indicate that bioreactors were not yet fully developed at the beginning of the trial. However, this was not detected in the TAN values, which were quite stable throughout the trial. Although all carrier media were used for six months before the trial started, it is possible that mixing and transferring might have disturbed nitrite oxidizing bacteria, which

are more vulnerable to changing conditions (Graham et al., 2007), and caused nitrite accumulation. This accumulation was higher in the FF group as compared to FM and MM groups, which is in contrast to other experiments that have compared similar carrier elements (Pedersen et al., 2015; Suhr and Pedersen, 2010). Fixed bed bioreactors are very susceptible to reactor dynamics, especially for the flow velocity (Kumar et al., 2011; Prehn et al., 2012). It is possible that the water velocity in our fixed bed reactors was not optimal and possibly water did not flow uniformly through all the filter media. Prehn et al. (2012) observed that when the water velocity is increased from 4.2 cm min^{-1}, which was the same velocity as in our system, to 66.7 cm min^{-1}, nitrification rates increased three-fold. In addition, the possibility of shunts in the FBBRs could have reduced the nitrification rates, because the by-pass flow may have decreased the active bioreactor surface area.

The nitrification rates measured in the laboratory were comparable to the observed nitrogen results from the water quality analyses, and both indicated that FBBR was less effective in the process. Sampling locations of the carrier media might have had some effect on the results, because MBBR has a unifying bacterial consortium throughout the reactor, while in FBBR, the bottom of the reactor can have different communities because of high substrate concentrations (Pérez et al., 2005). However, nitrification rates measured in the laboratory confirmed that nitrification in FBBR was did not work as effectively as in MBBR. Nitrification rates were very consistent in all MBBRs, demonstrating that MBBR is a very stable, reliable and maintenance-free bioreactor type to use.

5. Conclusions

Here, we demonstrated that nitrification bioreactor design affects RAS water quality, mainly through accumulation of solids and nitrification problems. When using two moving bed bioreactors, the amount of organic matter increased, while with the two fixed bed bioreactors, toxic nitrite accumulated in the circulating water. However, the drum filter compensates for the particle removal in the moving bed bioreactors. This study revealed that no single bioreactor type studied here is more beneficial than any other when rainbow trout growth and health is concerned. However, observed differences on the water quality may lead for selecting one bioreactor type over another. Solids retention capacity of FBBR may even make drum filters unnecessary, thus saving space and installations, which decreases the construction costs. On the other hand, FBBR require constant maintenance, which increases the operational costs. Maintenance free MBBR can save operational costs, but if solids accumulation is causing problems, additional solid treatment system might be needed. There can be also other aspects that can be dependent on the bioreactor type, which were not investigated in this experiment, one being formation of off-flavour compounds. In addition, when both reactor types are in use, changing sequence from FBBR followed by MBBR to MBBR followed by FBBR might highlight best features from both bioreactor types.

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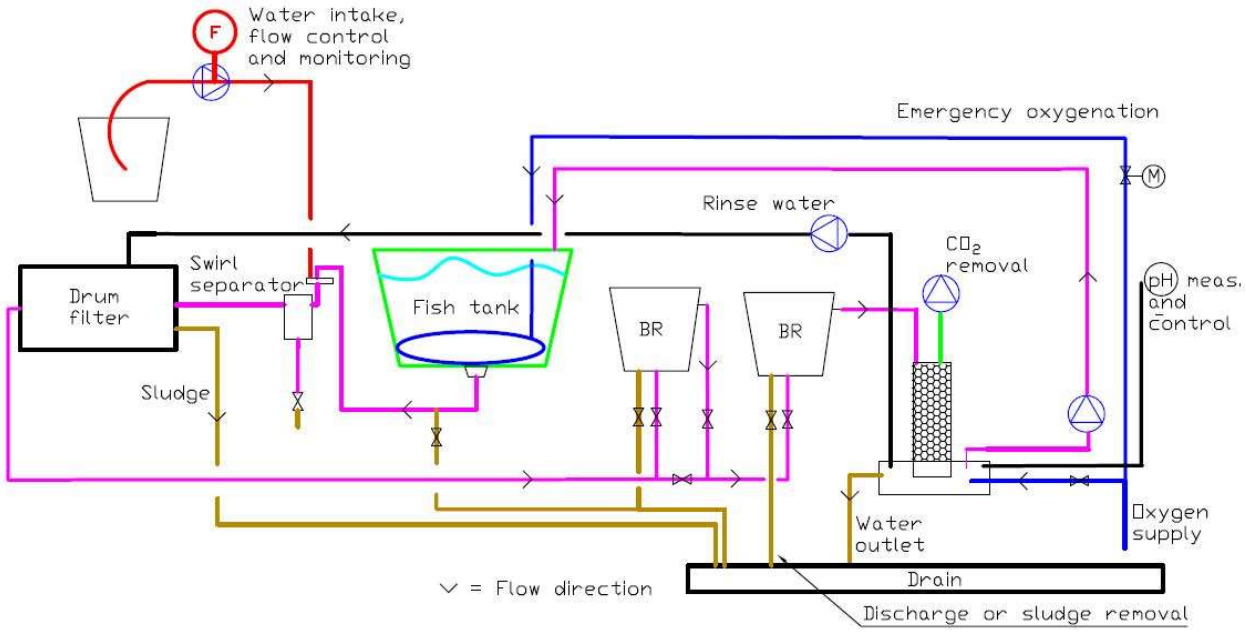
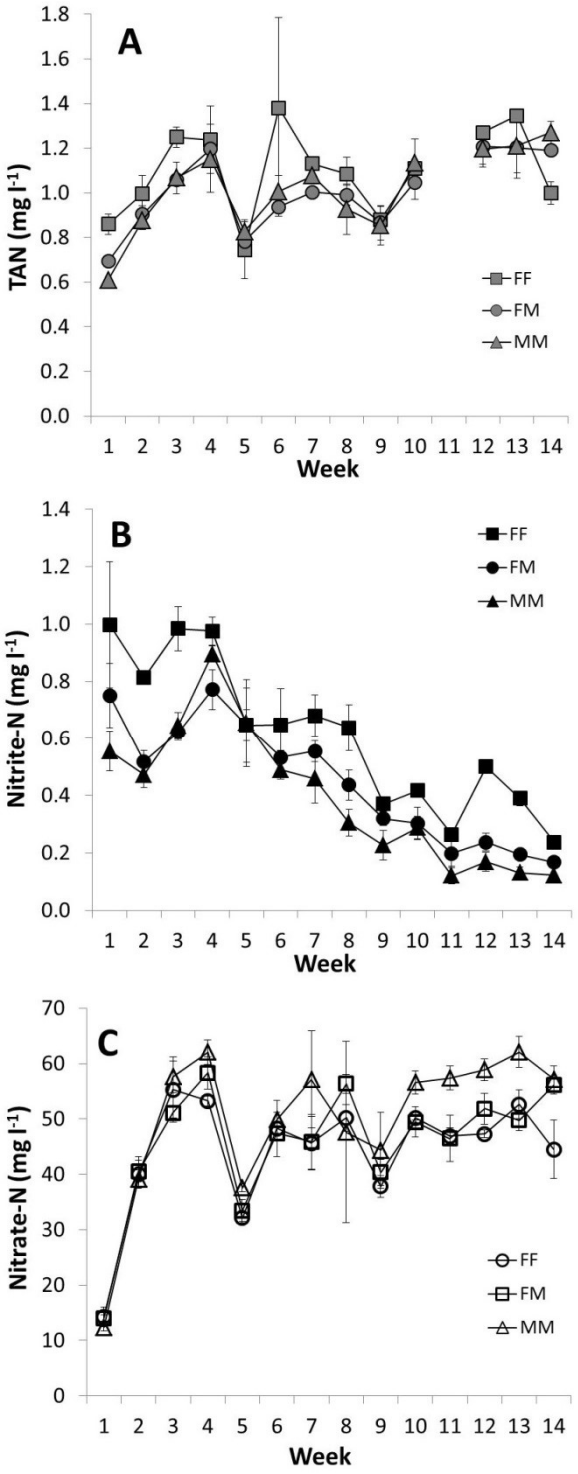


Figure 1. Schematic diagram of one RAS unit used in this experiment. BR = bioreactor, used as a fixed bed (FBBR) or moving bed bioreactor (MBBR).



516

517 Figure 2. Mean total ammonium nitrogen (TAN) (A), nitrite-nitrogen (B) and nitrate-nitrogen (C) values
518 of the three RAS bioreactor designs ± SD. FF = Two consecutive fixed bed bioreactors (n=3 at weeks 1-8
519 and n=2 at weeks 9-14), FM = Fixed bed bioreactor followed by moving bed bioreactor (n=3) and MM =
520 Two consecutive moving bed bioreactors (n=3).

Table 1. RAS operational design and rainbow trout (*Oncorhynchus mykiss*) rearing conditions in the trial, where different setups of fixed bed and moving bed bioreactors were studied.

Characteristics	Value	Unit
RAS unit (n=9)		
System volume	890	l
Tank volume	500	l
Relative water renewal rate	500	l kg^{-1} feed
Recirculation flow	15	l min^{-1}
Hydraulic retention time	5–8	d
Tank hydraulic retention time	33	min
Rearing conditions		
Fish density	19–82	kg m^{-3}
Feed quantity	0.22–0.45	kg d^{-1}
Average fish size	0.11–0.53	kg
Bioreactor (n=2)		
Bioreactor water volume	125	l
Bioreactor hydraulic retention time	8	min
Carrier media volume	66	l
Carried media area	49.5	m^2
Moving bed bioreactor air flow	15	l min^{-1}
Bioreactor hydraulic loading rate	436	l m^{-2} d^{-1}

Table 2. Mean rainbow trout (*Oncorhynchus mykiss*) feed conversion ratio (FCR), specific growth rate (SGR) (% bw d^{-1}) and thermal growth coefficient (TGC) (± SD) during the trial (1 = days 0–27, 2 = days 28–55, 3 = days 56–92, 4 = 0–92) of the three RAS bioreactor designs. FF = Two consecutive fixed bed bioreactors, FM = Fixed bed bioreactor followed by moving bed bioreactor and MM = Two consecutive moving bed bioreactors (n=3, except when marked in asterisk, where n=2).

	FCR				SGR				TGC
Treatment	1	2	3	4	1	2	3	4	4
FF	0.85 ± 0.03	0.95 ± 0.04	*1.13 ± 0.03	*0.98 ± 0.01	2.36 ± 0.05	1.56 ± 0.02	*1.03 ± 0.02	*1.59 ± 0.00	*2.20 ± 0.01
FM	0.81 ± 0.01	0.91 ± 0.02	1.07 ± 0.03	0.95 ± 0.01	2.44 ± 0.04	1.58 ± 0.03	1.11 ± 0.03	1.64 ± 0.01	2.28 ± 0.03
MM	0.81 ± 0.02	0.95 ± 0.08	1.05 ± 0.04	0.95 ± 0.02	2.41 ± 0.03	1.54 ± 0.08	1.12 ± 0.02	1.62 ± 0.02	2.22 ± 0.05

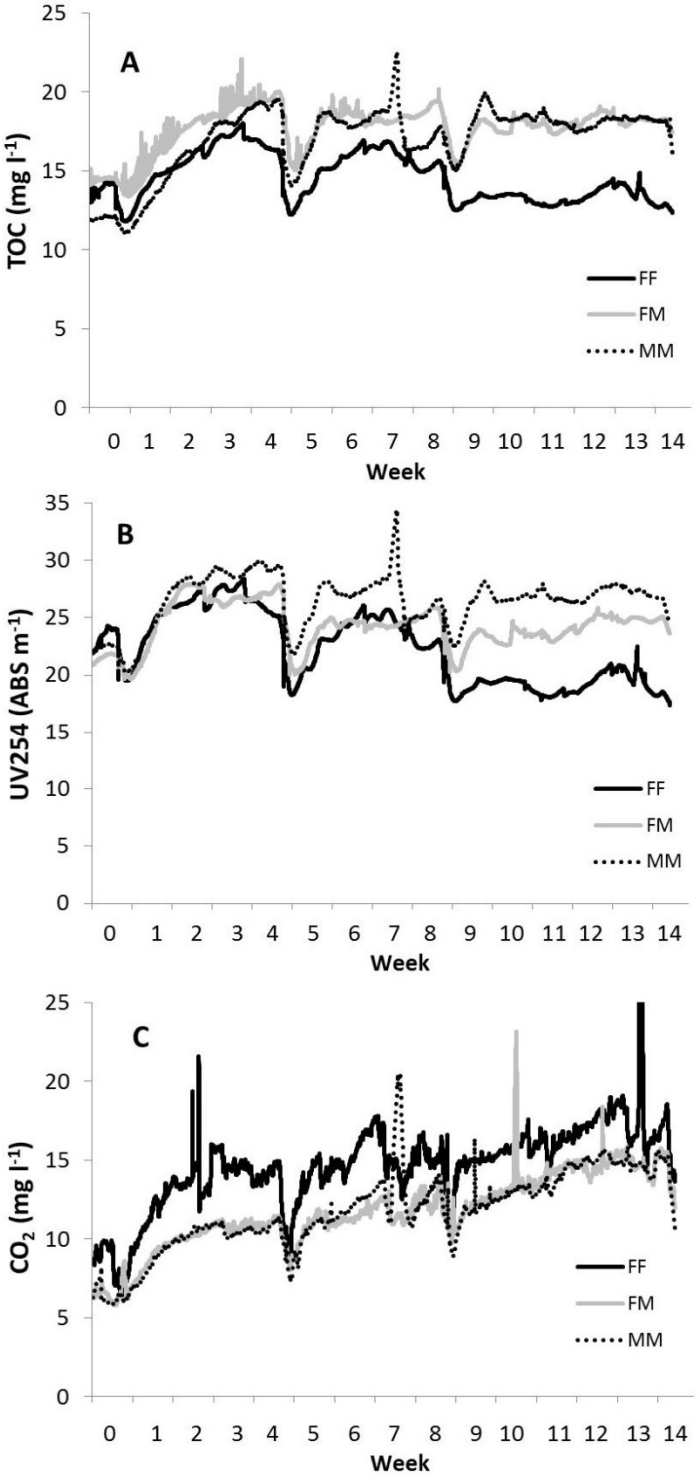


Figure 3. Mean total organic carbon concentrations (TOC) (A), UV254 absorbance (B) and carbon dioxide concentrations (C) measured online from the fish tank with UV/VIS spectrometer and CO_2 probe of three RAS bioreactor designs. FF = Two consecutive fixed bed bioreactors (n=3 at weeks 1-8 and n=2 at weeks 9-14), FM = Fixed bed bioreactor followed by moving bed bioreactor (n=3) and MM = Two consecutive moving bed bioreactors (n=3).

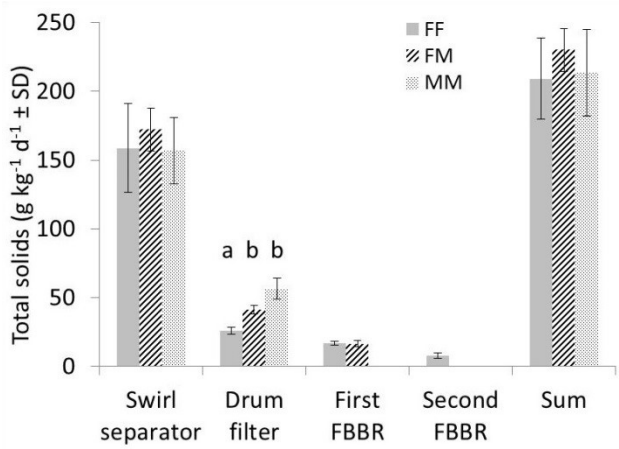
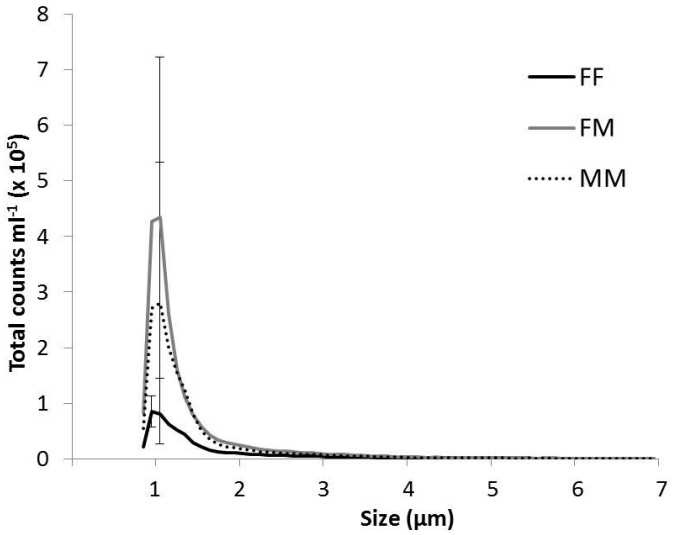


Figure 4. Total solids removed from different water treatment steps proportioned into daily feed intake of the three RAS bioreactor designs. FF = Two consecutive fixed bed bioreactors, FM = Fixed bed bioreactor followed by moving bed bioreactor and MM = Two consecutive moving bed bioreactors (n=3). FBBR = Fixed bed bioreactor. Mean values from two collection periods are presented (± SD). A significant difference between treatments in drum filter backwash water is marked by different letters ($p < 0.01$).

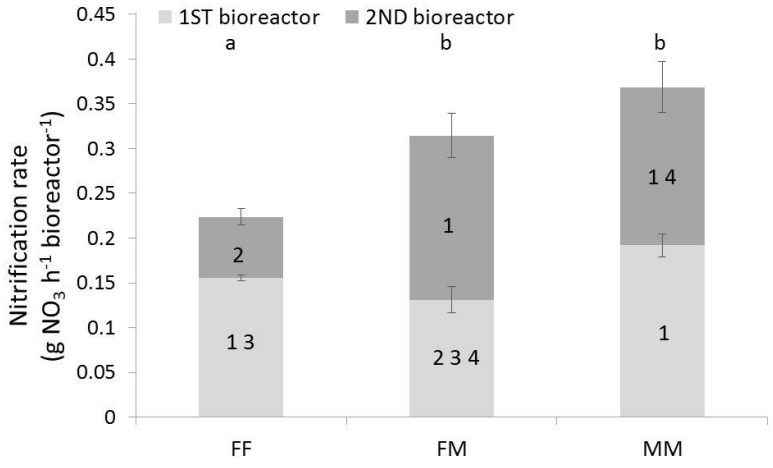
Table 3. Mean β-values, total particle counts, surfaces and volumes (± SD) at two sampling locations of the three RAS bioreactor designs. FF = Two consecutive fixed bed bioreactors (n = 3), FM = Fixed bed bioreactor followed by moving bed bioreactor (n = 3) and MM = Two consecutive moving bed bioreactors (n=3). A significant difference between treatments is marked by different letters ($p < 0.05$).

	Fish tank				After 2nd bioreactor			
	β	Total counts ($1.0x10^3$ pcs ml^{-1})	Total surface area (mm^2 ml^{-1})	Total volume ($1.0x10^{-3}$ mm^3 ml^{-1})	β	Total surface area (mm^2 ml^{-1})	Total counts ($1.0x10^3$ pcs ml^{-1})	Total volume ($1.0x10^{-3}$ mm^3 ml^{-1})
FF	3.7 ± 0.1	39.3 ± 8.3	0.7 ± 0.1	1.4 ± 0.1	4.1 ± 0.1^{a}	0.7 ± 0.2	40.5 ± 10.7	0.7 ± 0.2^{a}
FM	3.7 ± 0.2	45.8 ± 14.1	1.2 ± 0.4	2.0 ± 0.7	3.8 ± 0.2^{ab}	0.9 ± 0.5	35.6 ± 18.9	1.6 ± 0.5^{ab}
MM	3.6 ± 0.2	33.7 ± 13.9	1.0 ± 0.4	2.4 ± 1.0	3.6 ± 0.1^{b}	1.3 ± 0.1	40.9 ± 4.2	3.2 ± 1.0^{b}



549

550 Figure 5. Total particle counts of the three RAS bioreactor designs measured using the CASY cell counter.
551 FF = Two consecutive fixed bed bioreactors (n = 2), FM = Fixed bed bioreactor followed by moving bed
552 bioreactor (n = 3) and MM = Two consecutive moving bed bioreactors (n=3) ; ± SD of the most abundant
553 size classes, which were 1.0 µm for FF and 1.1 µm for FM and MM groups. There were no significant
554 differences between treatments.



555

556 Figure 6. Nitrification rate measured in the three RAS bioreactor designs measured using the stable
557 isotope labelling method. FF = Two consecutive fixed bed bioreactors (n = 2), FM = Fixed bed bioreactor
558 followed by moving bed bioreactor (n = 3) and MM = Two consecutive moving bed bioreactors (n=3). A
559 significant difference between treatments is marked by different letters and between different
560 bioreactors by different numbers ($p < 0.01$).