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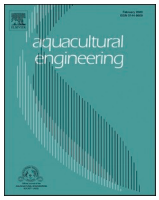

A case study comparing the addition of two different carbon sources in pilot scale RAS with trout with and without biofilters

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ABSTRACT

In this study, water quality and fish performance of traditional RAS with nitrifying biofilters were compared with systems operated under heterotrophic N assimilation (HET-N), a process where bacteria consume ammonium directly for growth and thereby remove dissolved N excreted from the fish, using three different modes of carbon addition. Using twelve identical pilot scale RAS, four treatment groups were established in triplicate: RAS with autotrophic biofilters (Control), RAS with autotrophic biofilters and acetate addition (BF RAS +Acetate), RAS without biofilter with acetate addition (BF RAS -Acetate) and RAS without biofilter and a biopellet reactor (Biopellet RAS). The nine RAS with carbon addition all had lower levels of nitrate and orthophosphate at the end of the trial compared to the three control RAS (approx. 70% less NO_3^- in the Biopellet RAS and 72% less PO_4^{3-} in BF RAS -Acetate). Without biofilters installed, both BF RAS -Acetate and Biopellet RAS maintained acceptable water quality parameters during their respective start-up phases and were fully developed in under 3 weeks. The addition of acetate to the water caused an expected formation of bioflocs in the systems, and a significant increase in bacterial activity and turbidity. Substantial feed spill was observed in RAS with acetate addition. The absence of bacterial accumulation and no increase in turbidity in the water in Biopellet RAS suggest that the processes primarily occurred within the reactor. The overall fish mortality was <1%, however, both types of RAS with acetate addition led to reduced fish growth (7.4–20%) compared to the control RAS and the RAS with biopellets. Biopellets were found to reduce dissolved N and P, and had a fast start up time without deteriorating water quality, thereby showing promising traits for use in RAS.

1. Introduction

At the heart of a modern RAS facility is the biofilter. It performs the essential function of converting the excreted toxic ammonia to less toxic nitrate (Malone and Pfeiffer, 2006; Ruiz et al., 2020). Due to low water exchange and long retention time of intensive RAS facilities, NO_3^- tends to build-up in the system and it's mostly controlled by the water exchange (Pedersen et al., 2012). While the nitrification process in the biofilter is relatively simple to start and operate, it is sensitive to a variety of different factors which can disrupt it. Among the key factors affecting the biological processes and thereby nitrification capacity are temperature, alkalinity, oxygen and organic matter. Alkalinity below 100 mg $CaCO_3$ l^{-1} has been shown to reduce nitrification capacity (Timmons and Ebeling, 2010) and likewise can low oxygen levels impair nitrification capacity (Chen et al., 2006; Suhr and Pedersen, 2010). The presence of excess organic matter can also have a detrimental effect on nitrification capacity (Ling and Chen, 2005; Michaud et al., 2006). This happens due to the growth of heterotrophic bacteria in the biofilter. Heterotrophic bacteria multiply at a much faster rate than autotrophs and under conditions with high enough organic matter will outcompete autotrophs for space and oxygen, leading to lower nitrification rates (Hagopian and Riley, 1998). On top of these challenges, the start-up time of autotrophic biofilters can take anywhere from a few weeks to several months (Lekang, 2007; Timmons and Ebeling, 2010), during which feeding of reared animals needs to be reduced, which has an economic impact on producers.

Furthermore, biofilters are also vulnerable to abrupt changes (increased feeding, salinity, exposure to disinfectant, etc.) which potentially can cause elevated levels of TAN or nitrite.

Heterotrophic nitrogen assimilation (HET-N) (Crab et al., 2007; De Schryver and Verstraete, 2009; Ebeling et al., 2006) is an alternative to autotrophic, nitrifying biofilters. In this process, heterotrophic bacteria assimilate ammonia directly for growth. This process is used in systems with bioflocs (Abakari et al., 2021; Crab et al., 2007; Hargreaves, 2013;

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Ogello et al., 2021; Yu et al., 2023). Heterotrophic N assimilation requires a relatively high carbon to nitrogen ratio (C:N ratio typically between 10 and 15), which can be achieved by adding a carbon source to the system (Avnimelech, 1999; Hargreaves, 1998). Normally, sugar, molasses, acetate or other low cost carbon sources, are added and dissolved in the water (Abakari et al., 2021; Saliling et al., 2007; Sharylo and Kovalenko, 2022; Zhang et al., 2016). Since heterotrophic bacteria grow faster than nitrifying bacteria (Hagopian and Riley, 1998), carbon addition leads to a faster start-up and turnover, and ideally stable and low dissolved N-concentrations. However, the addition of carbon and corresponding growth of heterotrophic bacteria leads to a large increase in oxygen consumption and increase in CO_2 (Avnimelech, 1999). Furthermore, as the bacteria grow as flocs in the water, turbidity and concentration of suspended solids tend to be very high, up to 500 mg l^{-1} (Ogello et al., 2021) compared to less than 20 mg l^{-1} in RAS (Schumann and Brinker, 2020). Systems relying on heterotrophic N-assimilation have predominantly been used for the production of shrimp, tilapia and catfish (Luo et al., 2017; Robles-Porchas et al., 2020), and have only recently been tested in the production of salmonids in RAS (Sharylo and Kovalenko, 2022).

Salmonids are raised in flow through systems and RAS, with high requirements for stable water quality, partly due to their susceptibility to increased loads of solids in the water (Schumann and Brinker, 2020). Recent studies have shown that rainbow trout (*Oncorhynchus mykiss*) tolerate elevated levels of organic matter in both acute (Becke et al., 2017) and prolonged exposure (Becke et al., 2018), even when exposed to simultaneous elevated levels of ammonia (Becke et al., 2019). Given that water quality is stable and the basic oxygen requirements are met, trout is considered a relevant (an interesting or a potential) candidate to investigate in RAS with carbon dosing.

An alternative to the easily available and biodegradable carbon sources used in biofloc systems are slow releasing solid carbon sources. These so-called biopellets can be composed of polyhydroxybutyrate (PHB) and other components and used as slow releasing fixed carbon media in designated reactors (Crab et al., 2007; Defoirdt et al., 2011). Unlike easily degradable carbon sources, PHB is hard to degrade and needs not to be added daily. The operation of a reactor with slow degradation PHB biopellets may also affect the turbidity and suspended solids as observed in biofloc systems. However, this remains to be tested.

The objective of this study was to investigate and compare conventional pilot scale RAS with RAS receiving different types of carbon sources. Carbon was added either as a dissolved compound (sodium acetate) or as biopellets (pine wood/PHB), and over a 4 weeks period, selected water quality parameters and fish performance were monitored.

2. Materials and methods

2.1. Experimental setup

To test and compare the effect of carbon implementation, 12 identical pilot scale RAS were divided into four different treatment groups in triplicates. A traditional moving bed biofilter was used in the control RAS (Control; Fig. 1A). One treatment group included acetate addition to a RAS with similar moving bed biofilters (BF RAS +Acetate; Fig. 1B). Another treatment group had no moving bed biofilter but only acetate addition (BF RAS -Acetate; Fig. 1C). In the final treatment group, the moving bed biofilter was replaced by a biopellet reactor (Biopellet RAS; Fig. 1D).

Each RAS consisted of a 500 l rearing tank (1 m length, 1 m width and 0.5 m high), a swirl separator fitted with a collector on the bottom for the removal of large solids and a 200 l sump. Six systems were fitted with a 90 l cylindroconical biofilter containing 40 L active, colonized RK BioElements (neutral RK BioElements, Denmark) with a specific surface area of 750 m^2 m^{-3}, operated as a moving bed biofilter (Fig. 1a). Three of these systems were used as control RAS, while acetate was added to the other 3 (BF RAS +Acetate). Another three RAS (Biopellet RAS) were fitted with a 20 l biopellet reactor (1.8 m high, 0.2 m diameter) operated as an up-flow reactor without aeration, with a flow of 600 l h^{-1} (Fig. 1d). 6 kg of biopellets (NP-BACTO-PELLETS, Tropic Marin, Germany) were added to each reactor. The biopellets had a composition of approximately 50% wood (pine) and 50% Polyhydroxybutyrat-

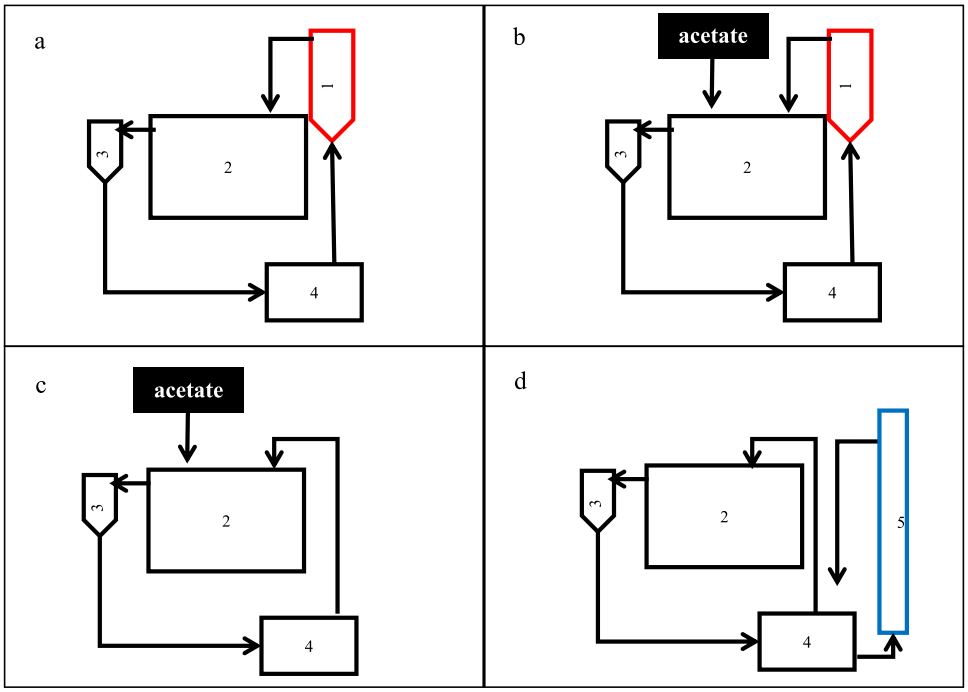

Fig. 1. Schematic representation of the systems used, with individual components. 1) biofilter 2) fish tank 3) swirl separator 4) sump 5) Biopellet reactor. Letter indicate the different treatment setups. a) Standard RAS systems used for control, b) Biofilter + acetate (BF RAS +Acetate), c) RAS without biofilter used for acetate only group (BF RAS -Acetate) and d) RAS without biofilter and with reactor for biopellets (Biopellet RAS). Arrows indicate water flows.

Hexanoat (PHB) (manufacturer communication), with approximately 0.827 g of COD per g of biopellet. The biopellets had a spherical form with a diameter of between 3 and 3.5 mm (Fig. 2). The remaining three (BF RAS –Acetate) RAS were operated with daily acetate addition without biofilters or biopellet reactors. In the biopellet RAS and the BF RAS -Acetate, the operational biofilters were disconnected prior to the start of the trial. A flow of 1500 l h^{-1} was pumped through the rearing tank in each of the 12 RAS. On systems fitted with biofilters, the water flowed from the sump to the biofilter and from the biofilter to the tank. In tanks without biofilters, the flow was directed from the sump to the tank.

Air stones were added to all sumps and tanks to provide airflow and ensure oxygenation and degassing of the system. Pure oxygen was added directly to the tanks and adjusted manually using a flow meter. Each tank was stocked with 8.08 (± 0.1) kg of rainbow trout, with an average weight of 430 g per fish. Feed was added using individual 12 h belt feeders. All RAS were fed 80 g (Efico E 920, Biomar, Denmark, 40.3% crude protein) on the first day of the trial (1% body weight), with the amount increasing by 1.1% daily, in order to maintain the proportion of feed to body weight throughout the trial. All tanks were fed below satiation levels in order to avoid feed spill from overfeeding. Every day, a quantity of 80 l of water was replaced in each of the 12 RAS, resulting in a feed loading ranging from 1 kg feed m^{-3} at the start of the trial to 1.35 kg feed m^{-3} at the end of the trial. The collectors below the swirl separator in each of the 12 RAS were emptied and checked for any uneaten feed pellets daily.

Basic water quality measurements were made every morning before any other daily routines. Oxygen, pH and temperature were measured in the swirl separator using a Hach HQ40d Portable Multi Meter (Hach Lange, USA). Ammonia, nitrite and nitrate were measured using MQuant tests (11117, 1110057 and 110020 respectively, Merck KGaA, Germany).

Sodium acetate anhydrous (0,7 g of C per g, Sigma-Aldrich, USA) was added daily to the BF RAS +Acetate and BF RAS -Acetate tanks in proportion to the feed given, with a calculated C/N ratio of 15–1 based on the daily production of dissolved N compounds. The feed was estimated to provide 15% of the dissolved carbon, while the sodium acetate provided the remaining carbon to achieve the desired C/N ratio. The acetate powder was placed in the belt feeder in order to distribute the dosing over the 12 h feeding period. Additional acetate was dosed during 7 days on BF RAS -Acetate due to higher ammonium levels, on a CN ratio of 16.9–1.

Sodium bicarbonate was added to the system in order to keep pH between 7.0 and 7.2, whenever necessary.

After four weeks, at the end of the trial, the biopellets from each reactor were collected and washed under running water before being dried in an oven at 60 °C for 48 h. After the drying process, the biopellets were weighed to estimate the amount of carbon used during the trial. Due to an unintended loss of biopellets from one of the 3 reactors to the sump, which were not possible to be collected, the data from only two reactors was used to calculate the carbon usage of biopellets. Fish performance was assessed by daily inspections of moribund fish and by estimating the biomass gain based on initial and final stocking biomass (Day 0 and Day 28).

2.2. Water sampling and analysis

Water samples were collected before the start of the trial and on a weekly schedule during the duration of the trial as in previous experiments (de Jesus Gregersen et al., 2021, 2020). A 2 L water sample was collected from each swirl separator (before any daily routines were performed) and homogeneous subsamples were collected from this sample. Samples were collected in the swirl separators in order to avoid disturbing the fish, which could result in resuspension of organic matter that could interfere with samples. Turbidity was measured with a Hach 2100Q (Hach Lange, USA) and % of UV transmittance (UVT) was measured using a quartz cuvette and UV spectrophotometer (Beckman DU® 530 Life Science UV/Vis Spectrophotometer, Bechman Coulter Inc, Indianapolis, USA) at 254 nm. Organic matter was measured as chemical oxygen demand (COD) and biological oxygen demand after 5 days (BOD_5). COD was measured following ISO 6060 (1989), as total (COD_{TOT}) and dissolved fractions (COD_{DISS}), where COD_{TOT} was measured in raw samples and COD_{DISS} was measured in pre-filtered samples (0,45 μm filter, Advantec® membrane filter, Toyo Roshi Kaisha Ltd, Japan). BOD_5 analysis were done according to ISO 5815–2 (2003) modified by the addition of allylthiourea (ATU) on raw samples.

Ammonium-N, Nitrite-N and Nitrate-N were measured according to ISO 7890–1 (1986), DS 223 (1991) and DS 224 (1975) respectively. Orthophosphate was measured according to ISO 6878 (2004).

Microbial activity was measured by an H_2O_2 degradation assay (Pedersen et al., 2019), expressed as the degradation rate constant (k, h^{-1}).

2.3. Data analysis

All data was analyzed in SigmaPlot 13.0 (Systat Software Inc., USA), checking for normal distribution (Shapiro-Wilk test) and equal variance (Brwon-Forsythe test). The results of the trial were compared at the last week of the trial, using a one-way ANOVA, followed by a Holm-Sidak test. Statistical differences between treatments were considered significant when $p < 0.05$. All results are presented as average ± standard deviation, $n = 3$.

Fig. 2. Biopellets used in the trial, before (left) and after the trial (right).

3. Results

During the 4 week experimental period of the trial, a total of two fish died, not related to a specific RAS treatment group. The temperature in the systems was 18 °C (± 1), while pH ranged from 7.2 to 8.5 and O_2 levels ranged from 70% to 114% (6.68–10.88 mg l^{-1}).

3.1. Dissolved nitrogen

NH_3/NH_4^+-N concentrations were low and stable throughout the trial in three of the RAS groups (ranging from 0.04 to 0.15 mg TAN l^{-1} in control RAS, 0.03–0.23 mg TAN l^{-1} in BF RAS +Acetate and 0.17–0.28 mg TAN l^{-1} in biopellet RAS, Fig. 3a)). The BF RAS -Acetate revealed a sharp increase in TAN during the first week, reaching up to 0.72 mg NH_4-N l^{-1}. The TAN concentrations were elevated for two weeks and then dropped to values similar to the other treatments, with no statistical differences found between treatments at the end of the trial (Fig. 3a, Table 1).

Nitrite levels remained below 0.27 mg l^{-1} in RAS with biofilters. The biopellet RAS showed the largest variations, with a large NO_2-N increase in the first week, up to 2.69 mg NO_2-N l^{-1}, then returning to the same level as the biofilter treatments in week 2 and remained stable for the rest of the trial (Fig. 3b). The BF RAS -Acetate had consistently higher nitrite levels than the RAS with acetate and biofilter with NO_2-N concentrations of 0.83 mg NO_2-N l^{-1} at the end (Table 1).

Nitrate levels increased steadily in the control RAS during the trial (Fig. 3c), as expected. Both acetate RAS groups (BF RAS +Acetateand BF RAS -Acetate) remained stable throughout the trial (from 22.5 ± 4.4 mg N l^{-1} to 25.,3 ± 1.2 mg N l^{-1} after 4 weeks and from 18.6 (± 8,2) mg l^{-1} to 18.8 (± 2.1) mg l^{-1} respectively). The biopellet RAS showed a different pattern of nitrate. During the first two weeks there was a significant decrease (from 24.2 mg NO_3-N l^{-1} to 3.09 mg NO_3-N l^{-1}), then followed by an increased to 11.7 mg NO_3-N l^{-1}.

3.2. Dissolved phosphorus

Orthophosphate followed a similar trend as nitrate, with a steady build-up in the control RAS, reaching a value of 2.76 ± 0,16 PO_4^{3-}-P mg l^{-1} at the end of the trial (Table 1). Both groups of acetate RAS showed a reduction of PO_4^{3-}-P in the first week, but then slowly rose throughout the trial. However, the BF RAS -Acetate had a larger initial decline (60% reduction from the start value compared to only a 28% reduction in the BF RAS +Acetate group) and a slower build-up during the rest of the trial. The biopellet RAS showed a large reduction of orthophosphate during the first two weeks similar to the nitrate concentrations (from a start value of 1.52 ± 0.14 mg l^{-1} to a value of 0.53 ± 0.1 mg l^{-1}) but after this point, PO_4-P rose to a final value of 2.19 (± 0.05) mg l^{-1}.

3.3. Turbidity and UV transmittance (UVT)

Turbidity remained stable in both the control RAS and the biopellet RAS throughout the trial (Fig. 3f). Both RAS groups with acetate experienced large and significant increases in turbidity. The BF RAS +Acetate reached a turbidity of 33.8 (± 6.6) FNU after 2 weeks, while the BF RAS -Acetate increased turbidity to 54.3 (± 4.3) FNU at week 3.

The water transparency measured as UVT was reduced in all 4 treatment groups during the experimental period. The control RAS dropped by 15.6%, the biopellet RAS by 24.4%, while the BF RAS +Acetate and BF RAS -Acetate were reduced by 37.8% and 52.3% point respectively (Table 1). Statistical differences were found between the

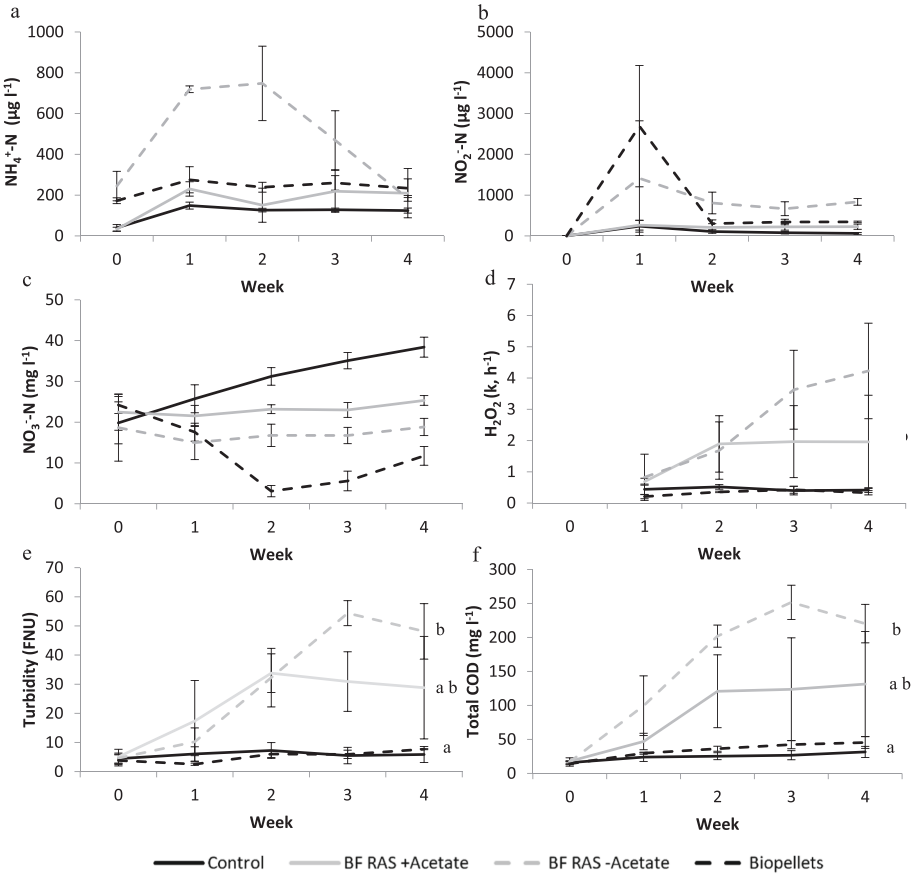

Fig. 3. Time series results of selected water quality parameters during the trial. a)NH_4^+-N b) NO_2-N c) NO_3-N d) Bacterial activity e) turbidity f) total COD. Letters indicate statistical differences between treatments. No differences between treatments where found in NH_4^+-N.

Table 1
Selected water quality values after four weeks of carbon addition to 12 RAS with rainbow trout at 15 °C.

Treatment	Control RAS		BF RAS +Acetate		BF RAS -Acetate		Biopellet RAS	
	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
Nitrate (mg NO_3-N l^{-1})	**38.4**	2.4	**25.3**	1.2	**18.8**	2.1	**11.7**	2.3
Nitrite (mg NO_2-N l^{-1})	**0.06**	0.02	**0.23**	0.06	**0.82**	0.08	**0.34**	0.02
Ammonium (mg NH_4-N l^{-1})	**0.12**	0.01	**0.21**	0.12	**0.18**	0.01	**0.23**	0.05
Orto-phosphate (mg PO_4^{3-}-P l^{-1})	**2.76**	0.16	**1.62**	0.04	**0.77**	0.2	**2.19**	0.05
Alkalinity (mg $CaCO_3$ l^{-1})	**48.3**	8.7	**548**	11.0	**601**	9.0	**147**	7.4
Turbidity (FNU)	**5.9**	2.8	**28.8**	17.6	**48.1**	9.5	**7.7**	0.2
UV transmission (%)	**61.3**	2.3	**36.9**	12.3	**24.6**	4.3	**50.9**	3.5
H_2O_2 (k, h^{-1})	**0.41**	0.08	**1.96**	1.49	**4.23**	1.5	**0.33**	0.07
BOD_{5Tot} (mg O_2 l^{-1})	**3.9**	1.03	**30.3**	24.97	**54.5**	4.8	**10**	3.3
COD_{Tot} (mg O_2 l^{-1})	**31.6**	8.2	**131.4**	77.3	**220.3**	28.3	**45.4**	8.6
COD_{Diss} (mg O_2 l^{-1})	**24.03**	4.05	**35.97**	2.4	**31.4**	8.6	**38.0**	5.6
COD_{Part} (mg O_2 l^{-1})	**7.6**	10.8	**95.4**	76.2	**188.9**	30.0	**7.4**	3.3

control RAS and the two acetate RAS groups, and also between the biopellet RAS and the acetate RAS groups.

3.4. Bacterial activity

The control RAS and the biopellet RAS revealed low and stable bacterial activity throughout the trial, with values of 0.41 and 0.33 h^{-1} respectively (Fig. 3d). Acetate addition increased the bacterial activity. The bacterial activity in the BF RAS +Acetate group increased 4.5 times compared to the control RAS, while the BF RAS -Acetate were found to have more than 10 fold increase in bacterial activity. The acetate RAS were found to have significantly elevated bacterial activity compared to control RAS and Biopellet RAS, and with substantial variation in the 3 RAS within the treatment group.

3.5. Organic matter

Total BOD_5 and total COD displayed similar trends as the bacterial activity (Fig. 3d and Table 1). The control RAS displayed a slightly lower increase compared to the Biopellet RAS. Both acetate RAS groups had significantly increased organic matter content during the trial, with the BF RAS +Acetate reaching approximately 7.7 and 4.2 times higher BOD_{5TOT} and COD_{TOT} respectively compared to control RAS and the BF RAS -Acetate reaching 13.9 higher BOD_{5TOT} and 7 times higher COD_{TOT} then the control (Table 1). Statistically significant differences were found between the acetate RAS group and both the Control and Biopellet RAS.

Dissolved COD increased over the 4 weeks, with the lowest values measured in the Control RAS and the highest in the biopellet group (24.0 ± 4.1 vs. 38.0 ± 5.6 mg O_2 l^{-1} respectively, Table 1). No statistical differences were found between the four RAS groups.

COD_{PART} followed the same trend as COD_{TOT} and made up most of the changes in COD. In particular, both Acetate RAS groups experienced large build-ups during the trial. Statistical differences were only found between the BF RAS -Acetate and both the control and biopellet groups.

3.6. Fish growth

During the duration of the trial, feed spill was observed in all six RAS receiving acetate. Fish from the BF RAS +Acetate grew 7.4% less than the control RAS, while fish from the BF RAS -Acetate grew 21% less over the 4 weeks (Fig. 4). Fish from the Biopellet RAS grew similar to the control RAS (2.5% more growth than the control). The BF RAS -Acetate had a significant lower weight gain compared to the growth of fish from the other three RAS Groups ($p < 0.05$).

3.7. Carbon used

The amounts of carbon used in the nine of the 12 RAS differed. In the BF RAS +Acetate group, an average total of 1957 g sodium acetate was added (1409 g of C), compared to 1999 g sodium acetate in the RAS without biofilters (1439 g of C). An average of 780 g biopellets (weight loss over the trial) were used in the Biopellet RAS, corresponding to 645 g of C.

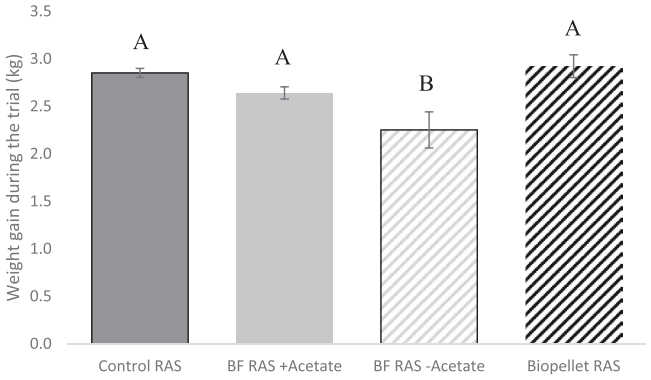

Fig. 4. Average weight gain by the fish in each treatment RAS group during the trial. Letters indicate statistical differences between RAS.

4. Discussion

4.1. Bacterial activity and organic matter build-up

The alternative metabolic pathways of heterotrophic bacteria compared to autotrophic bacteria have recently received attention (Deng et al., 2021; Huang et al., 2020; Wei et al., 2022).

The concept of promoting heterotrophic growth by adding carbon is well described (Crab et al., 2012; De Schryver and Verstraete, 2009). It is applied in the production of species like tilapia, catfish and different shrimp species (Avnimelech, 1999; Azim and Little, 2008; David et al., 2021; Khanjani et al., 2022; Ogello et al., 2021). However, while the use of Het-N is quite successful with this species, its use and potential/-compatibility with trout is unknown. Most species cultured under Het-N systems are very tolerant to changes in water quality and adapt very well to high levels of total suspended solids (Ogello et al., 2021). Furthermore, Het-N systems are generally applied in pond systems, where pumps are not used to generate flow, so the generation of flocs in a RAS design is not guaranteed.

Based on the operation of Het-N systems, we assumed that water quality would deteriorate with regard to organic matter in this system (Hargreaves, 2013; Ogello et al., 2021). However, the effects of using slowly degrading carbon in a pellet form were less clear, as it was possible that, as the bacteria consume the carbon, it would be released into the water phase and have the same effect as dissolved carbon

sources. The results obtained in this trial indicate otherwise. While the use of acetate as a carbon source resulted in the expected formation of bioflocs and significant increased levels of turbidity and organic matter, the systems fitted with the biopellets remained very similar to the control (Fig. 3 and Table 1). This was further supported by the bacterial activity results, where large increases in bacterial activity were observed in the water in the RAS treatments where acetate was added, but remained low in the Biopellet RAS throughout the trial (on par with control), suggesting that the majority of bacterial activity took place within the reactors. While organic matter was not measured in the reactors, the cleaning of the biopellets at the end of the trial revealed very large amounts of particulate matter built up within the reactor.

4.2. Nitrogen and phosphorus removal

Despite the promising results with the Biopellet RAS with regards to microbial water quality, N and P removal dynamics are more ambiguous. During the first two weeks of operation, the biopellets were fully fluidized. However, as organic matter (due to microbial growth and trapped organic matter) accumulated within the reactors, channelling started to develop. Thereafter, dissolved N and P concentrations increased again. There are different reasons for this; it could be due to a reduction of the available carbon source. When fully fluidized, each reactor contained 6 kg of biopellets in constant contact with the water, which provided enough carbon to convert all N and P to biomass. However, as the channelling developed, only the areas in contact with the flowing water could be used by bacteria, significantly reducing the available carbon. Another reason could be the partial anaerobic or completely anoxic areas within the reactor. Oxygen measurements at the outflow of the reactors showed that concentrations of O_2 were always above 80% due to the high flow rate and low hydraulic retention time (2 min approximately), however, due to the channelling, inner areas of the biopellets are likely to have generated very low oxygen areas.

The fast and significant reduction in NO_3 in the Biopellet RAS during the first two weeks of the trial suggests that, apart from NH_4^+ assimilation, denitrification also happened within the reactor, which could explain the peak in NO_2 during the first week (Tsukuda et al., 2015). The length of the trial and the issues with fluidization of the media complicate interpretation and conclusion and deserve further research. It is also likely that some nitrification took place during the trial, as there was an increase in NO3 at the end of the trial. The experimental setup did not allow us to quantify this particular autotrophic aerobic process, or distinguish other microbial processes (Het-N or denitrification) affecting the resulting nitrate concentrations.

On the other hand, acetate treated groups retained relatively stable levels of N and P throughout the entire trial. This suggests that both assimilation and nitrification were happening at the same time, as water changes did not result in any reduction in the N and P levels, which would be expected if all N and P were being uptaken by bacterial growth.

Available dissolved phosphate can potentially limit bacterial growth and activity (Dyhrman et al., 2007; Heisler et al., 2008; Paytan and McLaughlin, 2007; Sundareshwar et al., 2003) which may explain why nitrate was not completely removed after being reduced for 2 weeks.

4.3. Fish performance

A potential advantage of a Het-N RAS is the nutritional value of the bioflocs formed. This can be used as feed for the animals being produced, which can consume the flocs directly (Azim and Little, 2008; Khanjani et al., 2022; Ogello et al., 2021) or potentially be harvested and valorised. However, trout are unlikely to eat the bioflocs produced (not investigated in the current trial). During the trial, both acetate RAS treatments had lower fish growth compared to the Control RAS, with a large significant difference found between the control and the BF RAS -Acetate. This difference in growth originated from a lower feed intake in the tanks where acetate was added, which could be observed via the collection of feed waste. The exact cause for the lower feed intake is less clear. It is possible that the suboptimal water quality resulted in a lower appetite. Alternatively, the dimensions of the tank (only 50 cm of water column) coupled with the extremely high turbidity may have led to visual impairment and stopped the trout from consuming the feed before it exited the tanks (Hansen et al., 2013).

The Biopellet RAS experienced similar levels of fish growth compared to the Control RAS, indicating no impacts from the use of biopellets as a carbon source. PHB has even been shown to improve the survival of different species when incorporated into the feed (Duan et al., 2017; Hung et al., 2015; Laranja et al., 2014). It remains to be tested if such benefits can be obtained in RAS with PHB biopellet reactors.

4.4. Applied perspectives

Besides the negative implications on water quality and especially on fish growth, the use of sodium acetate was also more time-consuming, costly and cumbersome than using slow-releasing carbon from biopellets. The addition of acetate required daily measurements and adjustment of dosing in order to keep TAN low. It is likely that once the system reaches maturity, daily adjustments and maintenance would become easier. The biopellet reactor provided all carbon needed daily. It is likely that, for continuous operation, regular backwashing and refiling with new biopellets would be required. Apart from this, no other operation was found to be necessary.

The results obtained are promising regarding the use of slow-releasing carbon sources as alternatives to traditional biofilter media, with positive effects on the levels of N and P, a much faster start-up phase (compared to traditional biofilters) and without measurable significant negative effects on the water quality and, more importantly, on the fish.

The start-up of a traditional nitrifying biofilter is a slow process that can take several months (Lekang, 2007; Timmons and Ebeling, 2010). During this period, fish producers cannot feed the fish at optimum levels, resulting in a slower growth and decreased revenue. The use of reactors with solid carbon could offer a faster alternative to the start-up phase of biofilters, potentially gaining several weeks of additional feeding, which could offset the cost of the carbon.

Furthermore, while in the current study the biofilters were removed in order to study the extremes, in real-world application the reactors could be coupled to running systems with traditional autotrophic biofilters, providing both autotrophic and heterotrophic pathways.

Some challenges which still need to be addressed when using slow-releasing carbon in aquaculture include the potential need for extra oxygenation and the need for harvesting methods to remove excess biomass from the reactors, and changes in the reactor design may also be required in order to ensure proper fluidization (e.g. addition of a paddle stirrer). The long-term application of such reactors is also unknown.

Furthermore, while slow-releasing carbon matched control RAS in terms of water quality and fish performance, this type of treatment has additional costs compared to conventional water treatment processes.

5. Conclusion

This is one of the first studies to show that it is possible to convert a conventional rainbow trout RAS to Het-N assimilation without the use of a traditional nitrifying biofilter, through the use of different carbon sources, without compromising fish survival and reducing dissolved N and P in the water. The use of easily degradable carbon such as acetate, while resulting in the formation of bioflocs as intended, also resulted in deteriorating water quality and affected fish performance by significant reduced growth. In contrast, slow degrading carbon such as biopellets in a reactor, resulted in reduced levels of N and P, with none to negligible effects on water quality and equal fish performance compared to Control

RAS. Furthermore, biopellets produced a much faster start-up time compared to traditional autotrophic, nitrifying biofilters, which could have economic benefits to producers. We therefore consider biopellets as a prime candidate for further research into alternative ways of operating RAS systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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