Aquacultural Engineering 58 (2014) 52–58

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

Intensive rearing of cod larvae (*Gadus morhua*) in recirculating aquaculture systems (RAS) implementing a membrane bioreactor (MBR) for enhanced colloidal particle and fine suspended solids removal

A.B. Holan*, P.-A. Wold, T.O. Leiknes

NTNU Department of Hydraulic and Environmental Engineering, N-7491 Trondheim, Norway

ARTICLE INFO

Article history:
Received 4 January 2013
Accepted 3 October 2013

Keywords:
Cod larvae
MBR
Recirculating aquaculture system
RAS
Membrane filtration
Colloidal particles

ABSTRACT

Intensive rearing of Atlantic cod larvae (*Gadus morhua*) was investigated in a conventional recirculating aquaculture system (cRAS) and a membrane modified RAS (mRAS). Cod larvae are sensitive to water quality, and beneficial effects on growth and survival from enhanced removal of colloidal particles, fine suspended solids and nutrient reduction were expected. Membrane bioreactors (MBR) are a potential technology for advanced water treatment in aquaculture. The aim of this project was to assess the effect of an MBR system for enhanced treatment in RAS. A cRAS and mRAS treatment train were operated in parallel. In the mRAS Scheme 8.5% of the recycle stream was filtered through the membrane at any time. The mRAS scheme demonstrated a significantly lower turbidity and number of colloidal particles as compared to the cRAS scheme, as well as significantly lower bacteria concentrations and more stability. Overall a 13% higher cod larvae growth (weight, %) at 40 dph and 3.5% higher survival rate at 50 dph was measured in the mRAS scheme. Results show there is a great potential of implementing a membrane filtration system in aquaculture recycling systems.

1. Introduction

Capture production has been stable for the last 20 years while aquaculture production has seen an increase throughout the world (FAO, 2012), with a growing interest for recirculating aquaculture systems (RAS) (Martins et al., 2010). In Europe the most established RAS technology is found in The Netherlands and Denmark. Dutch RAS productions are typically indoors, nearly closed production of African catfish and eel, while in Denmark RAS is established for semi-closed, out-door production of on-growing stages of trout (Martins et al., 2010). Some of the developments that have been made in recirculating systems producing Arctic char in North America are described by Summerfelt et al. (2004a,b). Blancheton (2000) describes the development in recirculating systems for Mediterranean species, while Tal et al. (2009) reported the development of a fully contained land-based, marine RAS growing gilthead sea bream (*Sparus aurata*) from 61 g to 412 g in the USA. Colt (2006) documented the characteristics of toxicity testing and selection of water quality criteria for water reuse applications. He also documented how water quality criteria like content of fine solids, nitrogen and colour compounds, oxygen, carbon dioxide and heavy metals are developed and assessed their impact in reuse systems.

Assuming the recirculating system is well managed (Masser et al., 1999; Hjeltnes et al., 2012) there are several positive effects of recirculating systems compared to flow through systems. In RAS there is an opportunity to reduce the water consumption (Verdegem et al., 2006), conserve heat (no seasonal interruptions), establish an environmental control that can reduce the risk of disease and pollution problems, and optimize the growth rates and health of the fish by surveillance of the water quality (Blancheton, 2000; Timmons and Ebeling, 2007; Blancheton et al., 2009; Martins et al., 2010; Chiam and Sarbatly, 2011). Another important aspect of RAS is the establishment of microbial control in the cultivation tanks by stabilizing the substrate to bacteria ratio. Variable contents of microbe substrate induces a condition that triggers growth of fast growing opportunistic bacterial species, while stable concentrations of substrate to bacteria induces growth of a slow growing, stabile and a more beneficent bacterial community referred to as water maturation (Vadstein et al., 1993; Skjermo et al., 1997; Salvesen et al., 1999; Attramadal et al., 2012b). Blancheton (2000) found that the biofilter was the main source of bacteria in RAS. Depending on operating mode, a conditioned and well-functioning biofilter can therefore successively mature and be dominated by a large population of slow growing bacterial types. Under these

* Corresponding author. Tel.: +47 91683316.
E-mail addresses: astrid.buran@ntnu.no, terje-holan@ntebb.no (A.B. Holan).

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http://dx.doi.org/10.1016/j.aquaeng.2013.10.001

conditions, nutrients available for growth are consumed thereby preventing the opportunistic bacterial populations from growing.

In conventional RAS, biofilm processes are commonly used where biodegradable organic carbon and nutrients like ammonia and nitrite are oxidized by heterotrophic and autotrophic bacteria, respectively. Tal et al. (2003) characterized the different microbial communities associated with a moving bed bioreactor (MBBR) system used in a marine recirculating system and reported that both ammonia and nitrite oxidizers, *Nitrosomonas cryotolerans* and *Nitrospira marina* respectively, were found as well as a number of heterotrophic bacteria, including *Pseudomonas sp*. and *Sphingomonas sp.* Gutierrez-Wing and Malone (2006) reviewed the implication of the changing use of RAS and how this affects biofiltration research, where increased emphasis needs to be placed on the sizing criteria, and where acclimation problems in marine systems appear to justify the development of new acclimation procedures. Nitrogen (N) sources in the water have been identified coming from urea and feces, as well as organic debris from uneaten food and dead organisms (Timmons and Ebeling, 2007). According to Kristiansen and Cripps (1996) about 25% of total nitrogen is in a particulate form. Cripps and Bergheim (2000) reported that about 7–32% of the total nitrogen (TN) is in the particulate fraction with the rest in the dissolved form. Ammonia (NH_3) and ammonium (NH_4^+) exist in an equilibrium depending on pH, temperature and salinity (Bower and Bidwell, 1978; Johansson and Wedborg, 1980; Colt, 2006; Chiam and Sarbatly, 2011). The unionized form NH_3 can enter biological membranes such as fish gills because of the non-polarity and is therefore more toxic to aquatic species than the polar form NH_4^+. Eddy (2005) describes the general lethal concentrations with over 50% mortality (LC_{50}) for marine fish species to be 0.09–3.35 mg NH_3 L^{-1} (96 h LC_{50}). In general NH_3 concentrations for long-term exposure should be below 0.05 mg L^{-1} (Timmons and Ebeling, 2007). Solids in RAS are typically generated from excreted waste, dead and living bacteria and uneaten food. 11–38% of the total applied feed remains uneaten or is excreted by the fish (Chiam and Sarbatly, 2011), however, solids generation is very much dependent on several aspects such as feed quality and quantity, and feeding techniques (Cripps and Bergheim, 2000). Chen et al. (1993) characterized the suspended solids and showed that 95% of the particles by number will be included in a range up to 20 μm, and 80% to 90% of the total weight of solids (prefiltered to remove particles >130 μm) by volume will be included up to 35 μm. Quemeneur et al. (2001) showed that in a semi-closed aqua farming system over 90% of the particles by volume were smaller than 30 μm for on-growing basins, and in the nursery the significant amount of particles by volume were larger than 200 μm because of difficulties in swallowing such large particles, however by number 99% of the particles in the nursery were smaller than 1.5 μm.

Conventional water treatment systems in RAS strive to remove solids to prevent mineralization and production of smaller components (Chiam and Sarbatly, 2011). However, the systems currently in use only manage to remove particles larger than 40–60 μm, leaving the fine suspended solids (particles <35 μm) and the colloidal particles (<1 μm) in the system. Consequently, accumulation of particles is a challenge in RAS (Cripps and Bergheim, 2000; Davidson et al., 2009; Martins et al., 2010; Chiam and Sarbatly, 2011). Particles will dissolve into microbe substrate and open up for opportunistic growth (Attramadal et al., 2012c), cause increased amounts of nitrogen by mineralization and increase the biological oxygen demand (Chiam and Sarbatly, 2011). Furthermore, particles can affect nitrification kinetics (Zhu and Chen, 2001; Chen et al., 2006; Michaud et al., 2006; Guerdat et al., 2011), offer protection from disinfection (Hess-Erga et al., 2008; Hess-Erga, 2010), partially smother the gill and affect the gill function (Bullock et al., 1994; Timmons and Ebeling, 2007), clog the biofilters (Eding et al., 2006), and increase biofouling of the rearing system.

Since accumulation of substances as a function of reduced water refreshment is a challenge in RAS (Martins et al., 2010; Chiam and Sarbatly, 2011), application of membrane filtration technology for more advanced and efficient removal of fine suspended solids and colloids is a treatment strategy that may be one way to solve this problem. However, membrane filtration in RAS may be a challenge due to the potential of increased membrane fouling caused by the accumulation of colloidal particles on the surface of or within the membrane which will decrease the filtration flux over time. Membrane fouling and clogging can be controlled by operation below critical flux, periodic backflushing/backwashing and relaxation techniques, and air-scouring (Judd, 2006; Wu et al., 2008).

Treatment of industrial and municipal wastewater by membrane filtration in a membrane bioreactor (MBR) is increasing as the knowledge of cost effective processes increases (Judd, 2008; Lesjean and Huisjes, 2008). MBRs are commonly understood as the combination of biological treatment using activated sludge (AS) for biological removal of nutrients and filtration performed by membranes (AS-MBR) (Leiknes and Ødegaard, 2007) to achieve an advanced level of organic, ammonia and suspended solids removal. Research has recently been done to test the application of an alternative water treatment method combining a biofilter using the moving-bed-biofilm reactor with a submerged membrane reactor, forming a hybrid biofilm membrane bioreactor, BF-MBR (Leiknes et al., 2006; Leiknes and Ødegaard, 2007; Ivanovic and Leiknes, 2008; Sun et al., 2010). BF-MBR has the potential of utilizing the best characteristics of a biofilm process and membrane separation resulting in a compact, efficient particle removal system. In RAS the biofilter has often been designed using the moving-bed-biofilm reactor (Odegaard et al., 1999; Rusten et al., 2006) and as such a BF-MBR system is an interesting alternative for marine recycling systems.

From the literature review it is apparent that BF-MBR systems have not been tested for marine aquaculture application, though some work has previously been conducted to test the potential of integrating AS-MBR systems in marine RAS. However, this is not a fully established and commercialized practice to date. An AS-MBR in freshwater RAS was investigated in 2002 by Viadero and Noblet for removal of fine solids. They suggest membrane filtration for niche applications such as larval fish culture. A study by Pulefou et al. (2008) showed that using an AS-MBR treatment system in RAS created an effluent with turbidity of less than 0.5 NTU. A study by Sharrer and Summerfelt (2007) showed that the AS-MBR performed exceptionally well during operation, with nearly complete removal of $cBOD_5$, TSS and bacteria, and a biological treatment of nitrogen through nitrification/denitrification indicated consistent removal of total nitrogen. In a subsequent study permeate flow was found suited to be reclaimed in fish cultures to recycle alkalinity, salts, heat and water under biosecure conditions (Sharrer et al., 2010).

This study investigated the effect of an advanced system for enhanced colloidal particle, fine suspended solids and nutrient reduction by assessing a biofilm membrane bioreactor (BF-MBR) unit in a RAS water treatment system. The effect of the membrane treatment on number of colloidal particles, number of bacteria, turbidity and nutrient concentration was investigated, and the effect this had on the cod larvae growth and survival.

2. Methodology

2.1. Pilot plant configuration and components

Cultivation of Atlantic cod larvae (*Gadus morhua*) was investigated and conducted in two parallel pilot plant setups, a

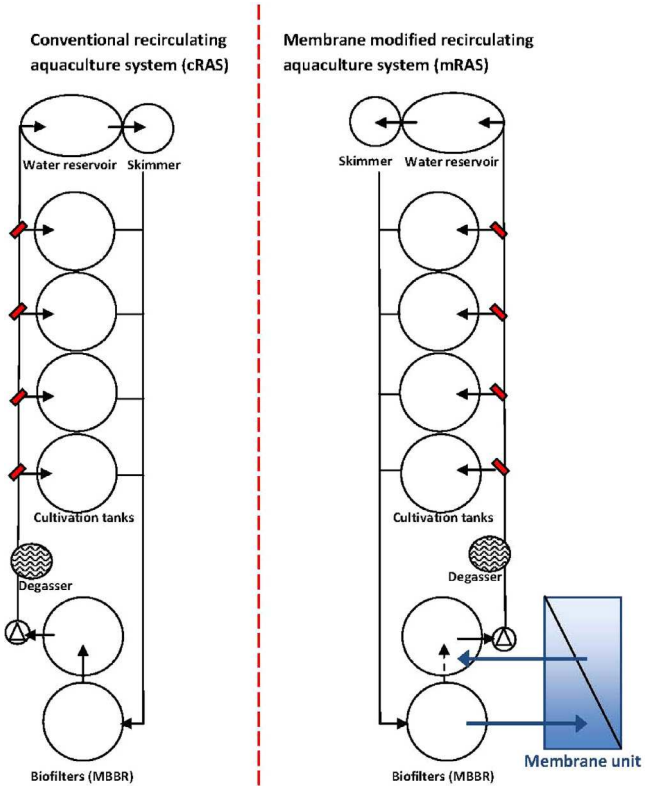


Fig. 1. Process configuration, system 1: conventional RAS, system 2: membrane modified RAS (mRAS).

conventional recirculating aquaculture system (cRAS) and a modified RAS connecting a membrane filtration unit between two biofilters (mRAS) (Fig. 1). The RAS pilot plants were operated with a recirculation rate of 89.3%, giving a recycle water flow rate of 12.7 L/min. The recycle flow passed from a water reservoir (160 L) to a skimmer (80 L) before entering two biofilters in series (267 L each), then to a degasser (50 L, vacuum operated) for removal of N_2 and CO_2 before being returned to the water reservoir. Four rearing tanks (100 L) in each treatment line were fed with the treated water with a water volume exchange rate set to 2–12 times per d^{-1} over a period of 50 days from hatching.

The membrane filtration unit consisted of two low pressure ultrafiltration modules operated in an outside-in mode in a submerged configuration with feed water from one biofilter and permeate discharged to the next biofilter. The biofilters were designed as moving-bed-biofilm reactors (MBBR), filled with biofilm carriers type K1 (Anox Kaldnes), with a filling fraction of 15% of the reactor volume, giving an area for biomass growth of 75 $m^2/m^3_{reactor\ volume}$. Each membrane module (PURON® polymer membranes from Koch Membrane Systems) was installed in a 30 L tank, and had a total area of 1.94 m^2 with the pore size of 50 nm. Continuous air-scouring (17 L/min) was applied, and a constant filtration flux of 33 L m^{-2} h^{-1} with alternating backwashing (3 times of 0.5 h d^{-1}) and relaxation (2 times of 0.5 h d^{-1}) was set. The flux was chosen based on what is commonly applied for commercial AS-MBR processes for municipal wastewater treatment (Judd, 2006), and studies on the development of BF-MBR processes (Ivanovic and Leiknes, 2012). This design treated the whole water volume 2.0 times d^{-1} or 8.5% at any time. The performance of the membrane system was determined by measuring the transmembrane pressure (TMP), monitored with a pressure transducer (Standard Genspec, 4–20 mA, ESI Technology). The set point for chemical cleaning of the membrane was at a TMP of 0.3 bars, only conducted on day 17 and 42 post hatching (ph). Chemical cleaning was accomplished by soaking the membranes with 40 g citric acid for 4 h, 0.5% hypochlorite for 8 h, and then 20 g of citric acid for 3 h. In the production period before the first chemical cleaning, the concentrate was diluted with 30 L fresh seawater on a daily basis. From then on the concentrate was completely replaced with fresh seawater one time per day.

2.2. Preparation of cod larvae and rearing regime

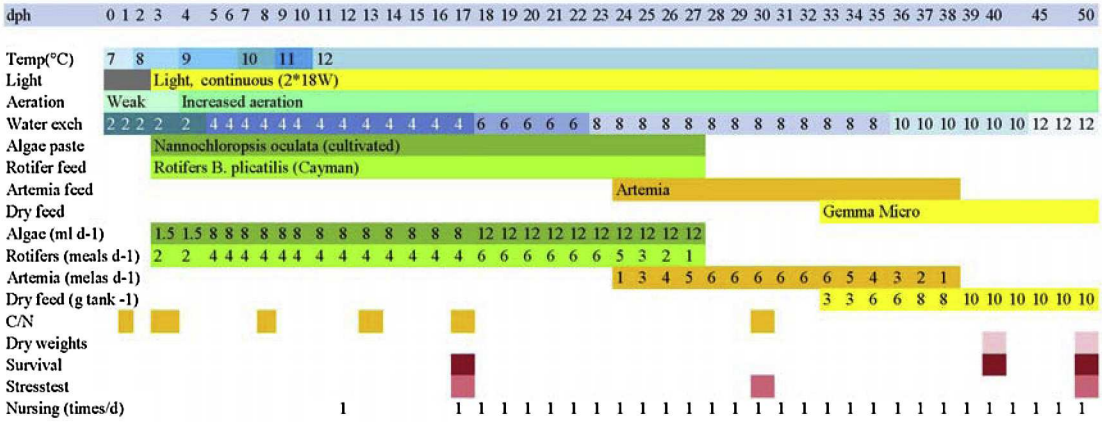
Atlantic cod eggs (*Gadus morhua*) received from Nofima marine national breeding station, Havbruksstasjonen i Tromsø AS, at 7.0 °C were disinfected in seawater containing glutaraldehyde (500 ppt) (Salvesen and Vadstein, 1995). The eggs were kept dark in an incubator tank. At −2 days post hatching (dph) the eggs were transferred from the incubator to rearing tanks of 100 L with a density of 100 eggs L^{-1}. At 0 dph 90% of the larvae were hatched. The temperature in the rearing tank water was 6.5 °C, salinity was 37 ppt at 1 dph, but was gradually reduced to 34 ppt at 4 dph, and 30 ppt at 9 dph. Elevated levels were again measured towards the end of the experiment.

The rearing regime is shown in Table 1. The fish larvae were kept in darkness the first 3 days of rearing, then in continuous light (2 × 18 W). Feeding of the cod larvae consisted of enriched rotifers (*Brachionus plicatilis*, Cayman), Artemia nauplii and formulated diet (dry feed). At day 3 dph algae paste (*Nannochloropsis occuluta*, Reed Mariculture) was added to the fish tanks to achieve a concentration of 1 mg C L^{-1} (1.5–12 ml d^{-1} from 3 to 27 dph). Rotifers were fed at tank concentrations of 5000–12000 rotifers L^{-1} (3–23 dph). The rotifers were cultivated in a continuous flow through system, fed yeast (Baker's yeast, *Saccaromyces cerevisae*), rotifer diet (0.5 ml 10^6 ind^{-1} d^{-1}) with addition of Lipid emulsion Easy DHA selco enrichment, 65% lipid content (0.2 μg emulsion ind-1, 2 h of incubation) (INVE, Belgium) before washed with seawater and added to a reservoir tank (250 L). From 24–34 dph the cod larvae were fed Artemia nauplii hatched from EG cysts (INVE, Belgium) and enriched with Multigain (Biomar) for 20 h before distribution to tank concentration of 2000–3000 L^{-1}. Feeding was conducted with a robot distributer applying rotifers 4–6 times d^{-1} and Artemia 6 times d^{-1}. From 33–50 dph the feeding robot distributed 3–10 g dry feed d^{-1} (Gemma Micro Diamond 300, Skretting, Norway) to each tank. Cleaning of the cultivation tank was performed on day 12 dph, 17 dph and then on a daily basis.

2.3. Analytical protocols

Water quality analyses for pH, temperature, salinity and dissolved oxygen (DO) were conducted regularly during the experiments. Number of bacteria was counted in fixed samples (formaldehyde, 2% final concentration that had been stored dark at 4 °C) using flow cytometry (Becton Dickinson FACScan) where Sybr Green I (nucleic-acid gel stain, Molecular Probes Invitrogen) was the fluorescent dye (Marie et al., 2005). Water samples for filtered total organic carbon (fTOC) measurements were immediately filtered through ignited (480 °C, 2 h) 47 mm GF/F (0.7 μm pore size) glass fiber filters (Whatman International Ltd., England) and stored frozen at −20 °C. Filtered total organic carbon (fTOC) concentration was measured by a Tekmar-Dohrmann Apollo 9000 TOC-analysator (Teledyne Tekmar, USA). Turbidity (NTU) was measured by a turbidity analyzer (2100N, HACH) and total ammonia nitrogen (TAN) was measured with a colorimeter (DR/890, HACH). Ammonia-N was calculated from TAN concentrations and adjusted for pH, temperature and salinity. Water samples for number of colloidal particles (30 nm to 1 μm) were analyzed by a NanoSight LM10 instrument (NanoSight, Amesbury, United Kingdom) immediately after sampling. All sampling from the experimental system were done using a filter tube (64 μm pore opening).

Table 1
The rearing regime for cultivation of Atlantic cod larvae (*Gadus morhua*).



At 50 dph the number of live cod larvae was counted giving the information of total survival. Survival at day 17 dph was calculated from daily mortality counts (17–50 dph).

For C/N analysis 12 larvae were randomly picked from each tank at days 1, 3, 8, 13 and 17 dph, given an overdose of tricaine methanesulfonate (MS222), rinsed in deionised water, and put in individual preweighted tin capsules for further analysis using a CHNSO analyzer (ECS 4010, Costech instruments, Elemental combustion system, Series number 260610079) measuring the carbon and nitrogen content. Larvae dry weight (DW) was estimated from the carbon content. A conversion factor of 2.34 for the carbon content was applied to calculate the dry weight (DW) (Reitan et al., 1993), meaning that the carbon content was 43% of the dry biomass. At day 40 dph, 40 larvae were randomly picked from each tank, given an overdose of tricaine methanesulfonate (MS222), rinsed in deionised water, put in individual preweighted tin capsules, dried (60 °C, 48 h) and weighted.

Specific growth rate (SGR (mg d^{-1}) was calculated from the average dry weight at time t, DW_t, and initial individual dry weight, DW_0, according to Eq. (1):

$$SGR(d^{-1}) = \frac{\ln DW_t - \ln DW_0}{t - t_0} \quad (1)$$

Despite cooling systems for temperature regulation, the temperature was 0.7 °C (average) lower in mRAS compared to cRAS. When comparing larval size at the same day degree (d°), the temperature difference effect on individual larval dry weight (average DW for each tank) was corrected for at each sampling time according to Eqs. (2) and (3) (Attramadal et al., 2012a):

$$\mathrm{LnDW_{corr,\,mRAS}} = \ln DW_{ind} - SGR_{mRAS} \times \frac{d^{\circ}_{average}}{{}^{\circ}C_{average,\,mRAS}} \quad (2)$$

$$\mathrm{Ln\ DW_{corr,cRAS}} = \ln DW_{ind} - SGR_{cRAS} \times \frac{d^{\circ}_{average}}{{}^{\circ}C_{average,cRAS}} \quad (3)$$

where $d^{\circ}_{average}$ was calculated from the difference between the actual d° in one system and the mean d° between the two systems at each sampling time.

3. Results and discussion

To evaluate the efficiency of the membrane to remove the smallest fraction of the particulates from the recirculating system, the level of turbidity and number of colloids (particles smaller than 1 μm) were investigated. Results of the analysis of the content of colloidal particles and turbidity for the duration of the test in both the cRAS and mRAS pilot plants are shown in Fig. 2. The number of colloidal particles (Fig. 2 a) and turbidity (Fig. 2 b) was significantly lower in the cultivation tanks for the mRAS alternative at the set filtration rate. Highest values measured during the experiment were 38% reduction in number of colloidal particles (25 dph) and 77% reduction in turbidity (30 dph). From the result it is evident that in the process configuration which includes the membrane filtration, an improved water quality is obtained with respect to turbidity and fraction of colloidal material.

The impact of the different water qualities on the cod larva survival and growth in the two parallel systems is shown in Fig. 3. The cod larvae survival rates were investigated after 17 and 50 dph while the growth (measured by weight) is seen from 1 dph to 40 dph. A better performance was observed for the mRAS configuration with 3.5% average higher survival rate, and a significantly better performance with 13% higher growth rates (by weight) at 40 dph. The lower turbidity and number of colloidal particles in mRAS could possibly explain the higher cod larvae survival rate and cod larvae size experienced in the membrane filtrated tanks since colloidal particles can affect the gills thereby reducing the gill performance (Bullock et al., 1994; Timmons and Ebeling, 2007).

However, these findings may also be explained by the microbial interactions in the cultivation tanks. An important aspect for the health and growth of the fish is the microbial environment in the cultivation tanks. Fast growing opportunistic bacteria populations are known to be both harmful and detrimental to the cod larvae, and preventing such blooms through controlling the microbial environment is an important operating parameter. Bacterial counts and change in bacteria concentrations over time were compared for the cRAS and mRAS systems. Results show that the number of bacteria in the mRAS system was generally lower (up to 80%) and with more stable concentrations compared to the cRAS system (Fig. 4). This applies to both the conditions in the cultivation tank as well as in the recycled water from the biofilters (intake water). From an operational point of view, bacterial blooms are to be expected after a change in type of feed. The response to change in feeding regimes is apparent in Fig. 4 with diet changes occurring on day 3 dph (from rotifer and algae) on day 24 dph (change to Artemia), and on day 32 dph (change to formulated dry feed diet). The resulting blooms are not as pronounced in the mRAS compared to the cRAS systems. At day 27 the bloom is in fact totally absent in mRAS while still high in the cRAS following the change to the Artemia diet. The change to dry feed diet at day 32 had the most impact on bacteria growth in both systems. This is probably a result of a higher organic loading and more bacteria substrate available in dry feed

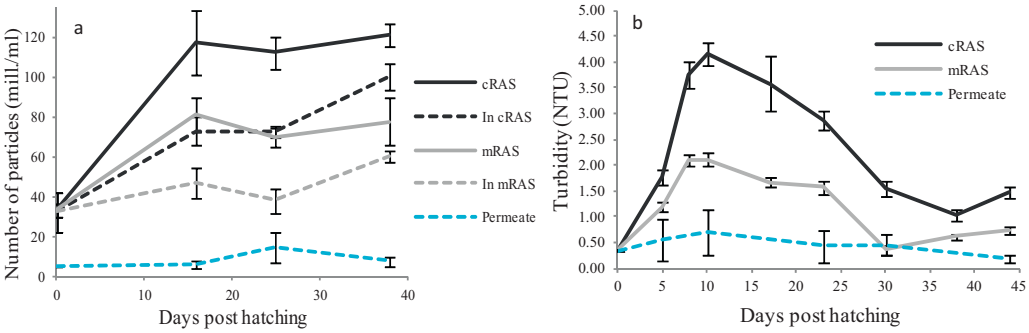


Fig. 2. Number of colloidal particles (mill./ml) (a) and turbidity (NTU) (b), cRAS and mRAS are water inside fish tanks ($n = 4$), In cRAS and In mRAS are intake water returning to the fish tanks from the biofilters ($n = 2$), blue bottom line is the permeate ($n = 2$). (For interpretation of the references to color in this text, the reader is referred to the web version of the article.)

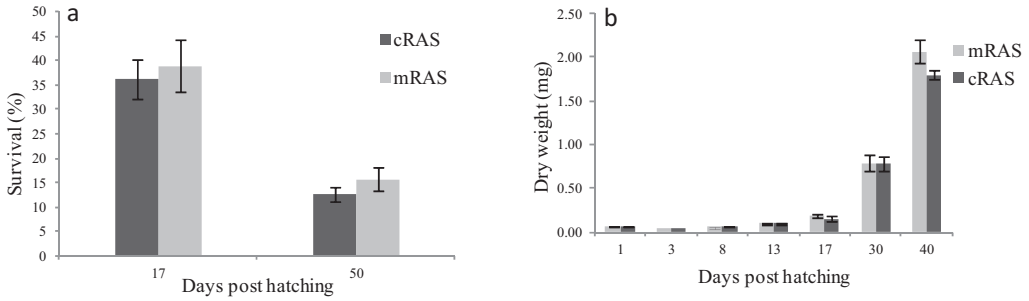


Fig. 3. Cod larvae survival (%) ($n = 4$) (a), and cod larvae weight (mg/larvae) ($n = 4$) (b).

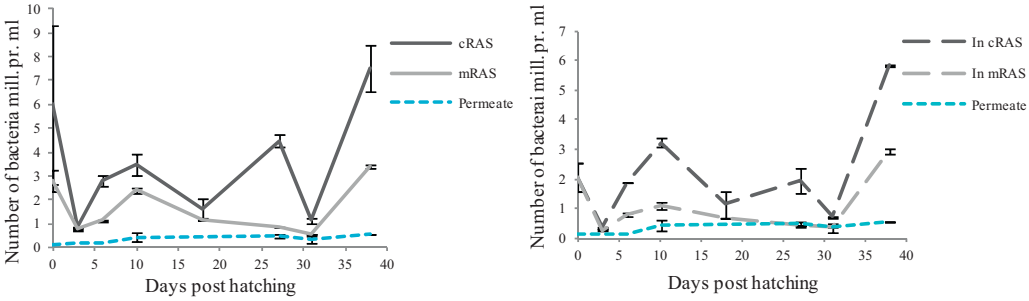


Fig. 4. Number of bacteria in rearing tanks (cRAS and mRAS) and in the permeate water ($n = 2$) (a), and number of bacteria in intake water from the biofilter to the tanks ($n = 2$), and in the permeate water ($n = 2$) (b).

compared to live rotifers and Artemia cells. However the bloom in mRAS is significantly lower than the bloom in cRAS. Stable concentration of substrate to bacteria is desired in cultivation systems and the findings shown in Fig. 4 correspond well with observations from growth rate and number of survival as discussed above.

Average physiochemical water quality parameters (pH, salinity, dissolved oxygen (DO) and temperature) from the whole experimental period are listed in Table 2. Except for the temperature, which was 0.7 °C lower in mRAS compared to cRAS, these parameters were essentially similar in the two water treatment schemes.

Table 2
Physiochemical water quality parameters (averaged from the whole experiment ± standard deviation).

Parameter	Cod larvae production	
	cRAS	mRAS
pH ± std	7.8 ± 0.01	7.8 ± 0.01
Salinity (ppt) ± std	32 ± 3.5	32 ± 3.3
DO (%) ± std	92 ± 0.95	93 ± 0.76
Temperature (°C) ± std[a]	11.6 ± 0.1	10.9 ± 0.1

[a] Averaged from 12 dph (when reaching stable temperatures)

With respect to other important water quality parameters, the concentrations of ammonia and fTOC (*i.e.* dissolved organic carbon) are shown in Fig. 5. The values for NH_3 concentrations observed in the mRAS system were significantly lower (up to 58% at 30 dph) compared to the values in the cRAS system. However, the values for NH_3 concentration are about 10 times lower than what is generally recommended for recirculating systems (<0.05 mg NH_3 L^{-1}) for both systems investigated and therefore did not likely affect the cod larvae growth and survival observed in this study. Parallel to this study the authors investigated the effect of integrated BF-MBR in RAS with respect to concentrations of N-compounds in the fish tanks, where the aim was to enhance autotrophic nitrification in the biofilter by reducing the organic load (i.e. C/N ratio) and heterotrophic competition (Holan et al., 2013). The values for fTOC concentrations observed in the mRAS system were lower (up to 52% at 9 dph) compared to the values in the cRAS system. Results show that the MBR setup was very efficient in reducing the organic content in the system, thereby stabilizing the substrate to bacteria

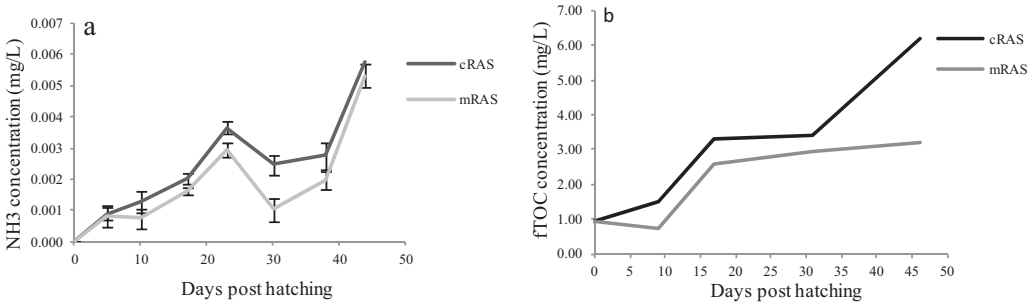


Fig. 5. (a) Ammonia (NH_3) concentration (mg L^{-1}) ($n = 4$) (b) and concentration of dissolved organic carbon given as filtrated total organic carbon (fTOC) in rearing tanks of cod larvae ($n = 2$).

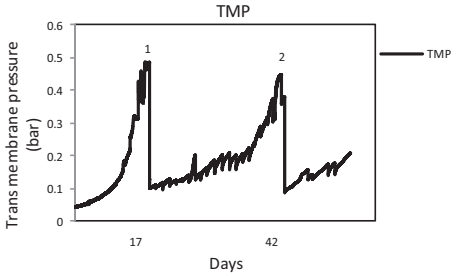


Fig. 6. Trans membrane pressure (bar).

ratio. The reduced amount of organic material found in the mRAS system will also impact the conditions for biomass growth in the cultivation tanks, which correlates with lower number of bacteria measured in the production tanks and intake water to the mRAS alternative (Fig. 4).

From responses observed in this study the potential benefits of a RAS system that integrates membrane filtration for advanced treatment of the recycle stream are apparent. However, a key aspect of any membrane filtration system is membrane fouling behaviour and control. In this study the membrane unit was monitored to assess the performance and behaviour of this separation process. Membrane performance is typically assessed by monitoring the change in transmembrane pressure (TMP), which is an indirect measurement of membrane permeability decline due to fouling phenomenon. The TMP measured over the duration of this study is shown in Fig. 6. During the experiment the membranes had to be chemically cleaned two times, seen as two peaks on the graph (Fig. 6). Operating conditions for the membrane filtration unit was based on conservative set points which are commonly used for MBR systems when applied in wastewater treatment. The fouling rates observed in this study are comparable with reported values from various studies on advanced wastewater treatment. It should be noted that a detailed investigation of the membrane performance was not the aim of this study, however, results from this study demonstrate that integration of a BF-MBR process in RAS is both feasible and practical. More detailed studies of membrane systems applied and optimal operating conditions are required for full-scale implementation and commercialization of mRAS solutions.

4. Conclusions

To meet the challenge of accumulated particles in RAS, this study investigated the effect of an advanced system for enhanced particle separation and nutrient reduction by assessing a membrane filtration unit combined with a biofilter unit in the water treatment system. The effect of the membrane treatment on number of colloidal particles, number of bacteria, turbidity and nutrient concentration was investigated, and how this may have impacted the cod larvae growth and survival. By filtrating the whole circulating water 2 times per day (or 8.5% at any time), the membrane filtration unit managed to remove colloidal particles and lower the turbidity to levels much lower than typically obtained in conventional recycling systems (e.g. up to 44% and 77% reduction for number of colloidal particles and turbidity, respectively). Bacteria concentrations were also significantly lower (up to 80%) and more stable in the membrane filtration system compared to the conventional system. These findings could explain the observed higher survival and growth rates in the intensive rearing of cod larvae found in this study. The ammonia level was up to 56% lower in the mRAS compared to the cRAS configurations, however, the ammonia values in both treatment schemes were well below toxic levels. The dissolved organic carbon (fTOC) level was up to 52% lower in the mRAS compared to the cRAS configurations and correlates to lower number of bacteria measured and a lower level of biomass growth in the mRAS alternative. The membrane filtration unit performed satisfactorily with relatively low fouling development and chemically cleaning only being necessary two times during the experiment. Further investigations in design and operation of the membrane filtration unit are required to optimize this component of the treatment scheme.

The potential benefits of a recycling system incorporating membrane filtration have been demonstrated. The mRAS configuration as applied in this study demonstrated a good removal of the colloidal particulate fraction, reducing accumulation of these particles in the system, improved removal of nutrients and demonstrated a maturation effect on the biofilter. The overall results obtained demonstrated healthier cod larvae with higher growth and survival rates achieved. However, further studies are desired for long term research on other species like salmon and wrasse, from larvae to on-growing stage, and in different designs and operating conditions of an mRAS concept.

Acknowledgments

This work was financed by RFF-MIDT, Norway (project no. 209048). The pilot plant setup and studies were conducted at the NTNU Centre for Fisheries and Aquaculture (Sealab), Brattørkaia, Trondheim, Norway. The authors like to thank Kari Attramadal (NTNU) for excellent help and discussions during the experiments, and for many useful discussions about microbial control, Morten O. Alver (Sintef) for all technical help with the automatic feeding system, and Keshuai Li for rotifer cultivation.

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