Assessment of a biofilm reactor coupled with membrane filtration for increased ammonia conversion, and reduced particle and nitrogen concentration in marine recirculating aquaculture systems (RAS)

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Sammendrag

Evaluering av en biofilmreaktor kombinert med membranfiltrering for økt omdanning av ammoniakk, og redusert partikkel og nitrogenkonsentrasjonen i marine resirkuleringssystemer for akvakultur (RAS).

Nitrifikasjonseffektiviteten i biofilter koblet til resirkuleringssystemer for akvakultur (RAS) avtar ettersom den organiske belastningen øker. Membranfiltrering i RAS ble undersøkt som en metode for å oppnå forbedret autotrof nitrifikasjon gjennom redusert organisk belastningen og heterotrof konkurranse. Denne metoden skal også redusere konsentrasjonen av totalnitrogen (TN) i systemet ved at den senker innholdet av partikler og dermed begrenser det påfølgende utslippet av næringsstoffer gjennom mineralisering. En sammenligning av en konvensjonell biofilm RAS (b-RAS) og en membran bioreaktor modifisert RAS (mbr-RAS) for produksjon av atlantiske torskelarver (*Gadus morhua*) ble gjennomført. Begge systemene ble operert identisk i form av vannutvekslingsvolum. Turbiditet, antall kolloidale partikler og konsentrasjonen av total ammonium nitrogen i fisketankene ble målt til å være signifikant ($p < 0,05$) lavere i mbr-RAS sammenlignet med b-RAS. Konsentrasjonen av nitrifikasjonsbiproduktene (nitritt og nitrat) var i den første perioden signifikant høyere i mbr-RAS, noe som indikerer økt nitrifikasjonsrate i biofiltrene. Etter tre uker var disse konsentrasjonen signifikant lavere i mbr-RAS, noe som sammenfalt med lavere målte verdier for turbiditet og antall partikler.

Summary

Nitrification efficiencies are reduced in biofilters in recirculating aquaculture systems (RAS) as the organic load increases. A membrane unit in RAS was investigated with the aim to enhance autotrophic nitrification in the biofilter by reducing the organic load and hence heterotrophic competition, and to reduce total nitrogen (TN) concentration by reducing the mass of colloidal particles and subsequent release of nutrients. Comparison of a conventional biofilm RAS (b-RAS) and a membrane bioreactor modified RAS (mbr-RAS) for production of Atlantic cod larvae (*Gadus morhua*) was conducted. Both

systems were operated identically in terms of water exchange volumes. The turbidity, content of colloidal particles and total ammonia nitrogen were measured to be significantly ($p < 0.05$) lower in the cultivation tanks for the mbr-RAS alternative. Initially the concentrations of nitrification by-products were significantly higher in the mbr-RAS indicating a potential increase in the nitrification rate in the biofilter. After 3 weeks the byproduct concentrations were significantly lower in the mbr-RAS, which coincided with the lower levels of turbidity and particles.

Introduction

There is an increasing interest in developing recirculating aquaculture systems (RAS) for enhanced water quality monitoring and control. In RAS, water treatment systems are always included to secure optimal water quality. Removal of nitrogenous compounds (N-compounds), organic compounds and particles are of utmost importance as they can be very detrimental to the species being cultivated. A conventional RAS always contains a biofilter for oxidation of N-compounds and organic constituents by autotrophic and heterotrophic bacteria, respectively. Elevated levels of nutrients could, however, be pronounced during certain situations, for example changes or increase in feeding regimes or failure of the mechanical filtration processes. Conventional designs of RAS commonly integrate modules for solids removal in the water treatment compartment. Solids are typically produced from excreted waste, microorganisms and uneaten food, and since it contains biodegradable organic compounds it is a source for microbial growth. Typically 11-38% of the total applied feed remains uneaten by the fish or is returned to the system as excreted waste (Chiam andSarbatly, 2011). Approximately 7-32% of the total nitrogen is found in this solid state Cripps and Bergheim (2000). Consequently, when particles mineralize and degrade, N-compounds will also be released and added to the system. This is however not the only source of nitrogen as ammonia is also directly excreted as a metabolic byproduct by aquatic species (Timmons andEbeling, 2007). The N-compounds are present in different forms and the impact in RAS depends on their state. Ammoniacal nitrogen (NH_3-N and NH_4^+-N) are of great concern as certain concentration levels can be toxic to aquatic species. In general lethal concentrations with over 50% mortality (LC_{50}) for marine fish species are 0.09-3.35 mg NH_3 L^{-1} (96h LC_{50}) (Eddy, 2005). In general lethal concentrations with over 50% mortality (LC_{50}) for marine fish species are 0.09-3.35 mg NH_3 L^{-1} (96h LC_{50}) (Eddy, 2005). Because of its non-polar nature, NH_3-N has the ability to enter biological membranes and is therefore of special concern to aquatic species. The amount of NH_3-N in the water, though, is dependent on ambient conditions as NH_3-N and NH_4^+-N exist in an equilibrium depending on pH, temperature and salinity (Chiam andSarbatly, 2011). Removal of ammoniacal nitrogen is commonly achieved through biological conversions (nitrification) in biofilters where autotrophic nitrifying bacteria oxidize NH_3-N/NH_4^+-N to the less toxic compound nitrate (NO_3-N) via the toxic compound nitrite (NO_2-N). Nitrate is diluted by adding make-up water to the system. Biofilters often applied in recirculating aquaculture systems are based on the moving bed bioreactor (MBBR) concept.

One of the challenges in RAS is that the autotrophic nitrifying biomass consists of slow growing bacteria species which, under certain conditions, can easily be outcompeted for space, substrate, and oxygen by heterotrophic biomass oxidizing available organic compounds (Chen, S. et al., 2006; Michaud et al., 2006; Pellegrin et al., 2009). This will subsequently affect the design and operation of the biofilter. Guerdat et al.(2011) found the volumetric TAN (total ammoniacal nitrogen, NH_3-N + NH_4^+ - N) removal rate (VTR) for three different types of biofilters was reduced by approximately 50% at elevated levels of biodegradable organic carbon as compared to normal operating conditions. This was also documented by Michaud et al. (2006). Consequently, a better removal of organic matter to reduce heterotrophic bacteria competition in the biofilter is beneficial to achieve improved nitrification in recirculating systems. Furthermore, the fraction of nitrogenous compounds coming from the mineralization (decomposition into the inorganic form) of particulate matter can be reduced by more efficient particle removal systems. In the conventional water treatment systems commonly used in RAS particles smaller than about 40 µm are not removed (Timmons andEbeling, 2007). Considering that 80 to 90% of the particulate matter in RAS is less than 35 µm in diameter (prefiltered to remove particles > 130 µm) (Chen, S. L. et al., 1993), there is a need to apply unit processes that can target these fractions. Accumulation of this fraction over time is a challenge resulting in an excess release of nitrogen compounds through mineralization. In these circumstances, ammonia concentrations could reach toxic levels in the rearing systems if the captured particulate matter is not removed from the system. The removal of fine particles, particularly the fine suspended solids (< 35µm) and colloidal fraction (< 1µm), is therefore important for a successful implementation of RAS.

Membrane filtration is a technology well suited for efficient removal of solids, including the colloidal fraction. Membrane bioreactors (MBR) have been developed for advanced wastewater treatment, combining a biological reactor with membrane filtration (Judd, 2008; Lesjean andHuisjes, 2008). MBRs are commonly understood as the combination of the activated sludge (AS) process for biological conversion of organics and nutrients together with a membrane filtration unit for removal of particles (AS-MBR) (Judd, 2006). A challenge often associated with MBR systems is the accumulation of particles of different sizes on the surface of or within the membrane module leading to fouling and clogging, and consequently reduced filtration flux (Bae andTak, 2005; Judd, 2004). Filtrating below a critical flux, periodically backwashing and air scouring, are means of reducing this problem to some extent (Judd, 2005; Wu et al., 2008).

An alternative to the AS-MBR is combining a biofilm reactor, e.g. a moving bed biofilm reactor (MBBR), with membrane filtration, forming a hybrid biofilm membrane bioreactor, BF-MBR (Leiknes andØdegaard, 2007). The benefit of a BF-MBR is that more specific and dedicated biofilm reactors can be designed which can target the needs in RAS while achieving enhanced removal of the colloidal particles. BF-MBR also works in a low solids environment which potentially is beneficial for the design and operation of the membrane unit (Leiknes and Ødegaard, 2007). From the literature review it is apparent that BF-MBR has not previously been tested for marine aquaculture systems, although some work has previously been conducted to test the potential of integrating the AS-MBR in marine RAS (Pulefou et al., 2008; Sharrer et al., 2010; Sharrer et al., 2007; Viadero Jr andNoblet, 2002).

The aim of this study was to investigate the potential of BF-MBR technology to improve the water quality in RAS with respect to concentrations of N-compounds in the marine fish larvae culture system. A focus has been on combining a biofilm reactor with a membrane filtration unit to increase nitrification in the biofilters through reduced C/N ratio, and to reduce nitrogenous compounds in the system by reducing the overall accumulation of fine solids and colloidal particles. The potential positive impacts on water quality in a BF-MBR modified RAS (mbr-RAS) cultivating Atlantic cod larvae (*Gadus morhua*) was assessed in a pilot plant study and compared to a conventional biofilm RAS (b-RAS). Nitrification kinetics was not a focus in this particular study. Parallel to this study the authors investigated the effect of integrated BF-MBR in RAS on tank microbial composition, cod larvae performance (Holan, A.B. et al., 2014; Wold, P.-A. et al., In prep; Wold, P. A. et al., 2013), and membrane performance and fouling behavior (Holan, A.B et al., 2013).

Methods

Process configuration

A pilot recirculating aquaculture system (RAS) plant for cultivation of Atlantic cod larvae (*Gadus morhua*) was built, consisting of two parallel RAS systems. One system contained only a biofilm reactor (b-RAS, system 1) and the other was modified to include a membrane filtration unit treating water from one bioreactor, forming a membrane bioreactor RAS concept (mbr-RAS, system 2), figure 1. In each treatment system, a water flow of 12.7 L/min was fed to two biofilm reactors (moving bed biofilm reactors, MBBR) in series (267 L each) filled with biofilm carriers type K1 (Anox Kaldnes), followed by a degasser (50 L, vacuum operated), and connected to a water reservoir (160 L), and a skimmer (80L). From this flow water was led to the fish tanks (100 L, 4 parallel tanks). In the mbr-RAS a submerged low pressure ultrafiltration membrane filtration unit was installed (PURON® polymer membranes, Koch Membrane Systems) with a total surface area of 1.94 m^2, nominal pore size 50 nm, in a 60L tank. The membrane tank received feed water from one biofilter (BF1), discharging permeate water to the downstream biofilter (BF2), while the retained concentrate in the membrane tank was discharged and replaced with fresh seawater (60 L) into the production system one time per day. This corresponded to 98% recovery. To match, the exact same volume (60 L per day) was also replaced (continuously) in the b-RAS treatment system. The MBBR had a filling fraction of 15 % of the reactor volume giving an area for biomass growth of 75 $m^2/m^3_{\text{reactor volume}}$. The membrane operating mode consisted of; continuous air-scouring (17 L/min), constant filtration flux of 33 L m^{-2} h^{-1}, and with alternating cycles of backwashing (3 times of 0.5h d^{-1}) and relaxation (2 times of 0.5hd^{-1}). This design treated the whole water volume 2.0 times d^{-1} or 8.5% of the water flow (12.7 L/min) at any time. The transmembrane pressure (TMP) was monitored with a pressure transducer (Standard Genspec, 4 – 20mA, ESI Technology) giving indications of membrane performance and time for cleaning. The set point for membrane cleaning was at TMP = 0.3 bars, and was conducted twice during the test on day 17 and 42 post hatching (ph).

The duration of the experiment was 50 days, and the feeding regime of the cod larvae consisted of enriched rotifers at 3 days post hatching (dph) (*Brachionus plicatilis*, Cayman.), Artemia nauplii at 24 dph and formulated diet at 33 dph (dry feed, Gemma Micro Diamond 300, Skretting, Norway). From 3 dph algae paste (*Nannochloropsis occuluta*, Reed Mariculture) was in addition added to the fish tanks (green-water). The total feeding regime is fully explained in Holan et al. (2014).

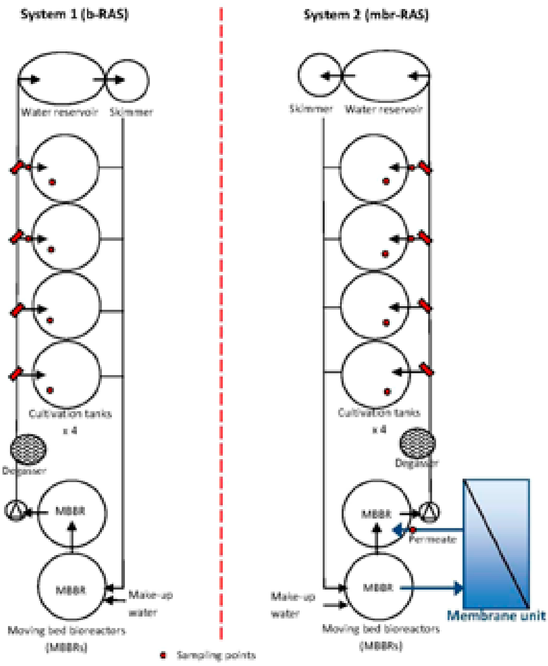

Figure 1. Process configuration: System 1: conventional biofilm RAS (b-RAS), System 2: membrane bioreactor RAS (mbr-RAS).

Physiochemical water quality parameters

The physiochemical water quality parameters were measured regularly during the experiment with the methods as described in table 1. All analysis/measurements are done according to the procedures supplied by the manufacturer.

Unionized ammonia-N (NH_3-N) was calculated from total ammonia nitrogen (TAN) concentrations and adjusted for pH, temperature and salinity. Water samples for filtered total organic carbon (fTOC) measurements were immediately filtered through ignited (480 °C, 2h) 47 mm GF/F (about 0.7 µm pore size) glass fiber filters (Whatman International Ltd., England) and stored frozen at -20°C.

Parameter	Instrument
pH, temperature	VWR pH10 (pen)
Salinity	Refractometer
Dissolved oxygen (DO)	YSI ProODO
Filtrated total organic carbon (fTOC)	Tekmar-Dohrmann Apollo 9000 TOC-analysator (Teledyne Tekmar, USA)
Turbidity (NTU)*	Turbidity analyzer (2100N, HACH)
Total ammonia nitrogen (TAN), nitrite (NO2) and nitrate (NO3)*	Colorimeter (DR/890), HACH)
Number of colloidal particles (30nm to 1µm)*	NanoSight LM10 (Amesbury, United Kingdom)

* Prefiltered before analyzing (64 µm pore opening)

Table 1. Physiochemical water quality parameters and method / instrument applied.

Statistics
Mean ± standard deviation (std.) is presented (n = 4 or n=2) and statistical analysis was performed at the 95% confidence level ($p < 0.05$) in IBM® SPSS Statistics 19 software. Data were tested for homogeneity of variance using a Levene test. To compare two means the group data was statistically tested using independent student`s t-test. Samples from the parallel fish production tanks were treated as replicates, although RAS were not replicated.

Results and discussions
Placing a membrane unit in the recirculating treatment system, as in the proposed MBR concept, aimed to enhance the nitrification processes in the biofilter by reducing the organic load and C/N ratio and thus autotrophic competition. Furthermore, membrane filtration was expected to reduce the overall concentration of all N - compounds in the system due to reduced solids accumulation causing mineralization and nutrient release. The potential of MBR technology to improve water quality in RAS was evaluated by analyzing the fate and removal efficiency of solids, particularly the fine and colloidal fraction, and the conversion and concentration of N-compounds. The results were compared between the two treatment systems, b-RAS and mbr-RAS. Statistical data from the study was carefully interpreted since the treatment system was not replicated. However, the main focus of the experiment was the potential effect of the MBR unit on the water quality with respect to concentrations of N-compounds in the fish tanks. A benefit of using one single treatment system will enable the same basal water quality to the four fish tanks, which may not be achieved by using replicate treatment systems (one for each tank) due to RAS-to-RAS variation (Terjesen et al., 2013).

Physiochemical water quality
The water quality in the production tanks was monitored over the length of the experiment. Table 2 gives an overview of the average values measured for the different physiochemical parameters in the production tanks. Stabile conditions were maintained in the pilot plant during the experimental period and with very similar conditions in the two parallel treatment systems.

Fate and removal of solids
Turbidity and number of colloidal particles were measured in water samples taken directly in the fish cultivation tanks. The number of colloidal particles was also measured in the treated feed water recycled back to the cultivation tanks. The development of the turbidity and content of colloidal particles in the production tanks for the b-RAS and mbr-RAS are presented in a parallel study (Holan, A.B. et al., 2014). Comparison between b-RAS and mbr-RAS for a specific period (days post hatching (dph)) is shown in table 3. For the number of colloidal particles in the fish

Parameter	Cod larvae production	
	b-RAS	mbr-RAS
pH ± std	7.8 ± 0.01	7.8 ± 0.01
Average salinity (ppt) *	32	32
do (MG/l) ± std	92 ± 0.95	93 ± 0.76
Temperature (ºC) ±std **	11.6 ± 0.1	10.9 ± 0.1

* n = 1
** Averaged from 12 dph (when reaching stable temperatures)

Table 2. Physiochemical water quality parameters in cod larvae production tanks (averaged from the whole experimental period with standard deviation) (n=4).

Parameter	Average ± std b-RAS / Average ± std mbr-RAS	Reduction (%)	Sampling point	Period (dp)
Colloidal particle (millions/ml)	96.0 ± 6.9	32	Fish tanks	0-38
	65.5 ± 5.4			
Colloidal particle (millions/ml)	69.5 ± 4.2	36	Treated feed water	0-38
	44.5 ± 2.7			
Turbidity (NTU)	2.39 ± 0.20	48	Fish tanks	0-38
	1.25 ± 0.09			

Table 3. Percentage reduction in turbidity and content of colloidal particles in b-RAS and mbr-RAS fish tanks (n = 4) and treated feed water (n = 2) (averaged values from a specific period with standard deviation).

Parameter	Average ± std b-RAS / Average ± std mbr-RAS	Average reduction (%)	Sampling point	Period (dph)
TAN (mg/L)	0.09 ± 0.01	26	Fish tanks	0-38
	0.07 ± 0.01			
fTOC	2.29	21	Fish tanks	0-31
	1.80			

Table 4. Total ammonia nitrogen (TAN) (n=4) and filtrated total organic carbon (fTOC) (n=2) in b-RAS and mbr-RAS, and the percentage reduction between the two treatments b-RAS and mbr-RAS (averaged from a specific period with standard deviation).

tanks and treated feed water, a reduction of 32% and 36% were measured, respectively, and a reduction for turbidity in the fish production tanks was measured to be 48%. Since nitrification efficiency was found to be depleted with increasing C/N ratio in the environments (Guerdat et al., 2011; Michaud et al., 2006), the effect of these findings on ammonia concentrations in the two treatment systems was further investigated.

Ammonia conversion

To assess the ammonia conversion efficiency, TAN, NO_2-N and NO_3-N were measured in water samples taken directly in the fish cultivation tanks and from the treated feed water recycled back to the cultivation tanks. To insure proper mixing in the tanks, air was applied from the central bottom. Samples were taken from a fixed position between the tank wall and the center outlet. Analysis of the total ammonia nitrogen (TAN) and filtrated total organic carbon (fTOC) revealed 26% lower average concentration of TAN and 21% fTOC in the mbr-RAS fish tanks compared to the b-RAS fish tanks for a specific period (0 – 38 dph), table 4. The difference was significantly lower for TAN, however for fTOC the number of replicate samples were 2 (n=2) and no statistical analysis was performed.

A more effective ammonia conversion was possibly taking place in the system containing the membrane filtrating unit. To asses this hypothesis, the nitrification process was further investigated by looking at the concentrations of ammonia, NH_3-N, the nitrification intermediate, NO_2-N, and nitrification end-product, NO_3-N, in the system over time (Figure 2a - c, respectively). At 17 days post hatching (dph) the concentration of NH_4-N was significantly lower, and the NO_2-N and NO_3-N was significantly higher in the fish tanks in the mbr-RAS compared to b-RAS, supporting the hypothesis of higher degree of ammonia conversion to NO_2-N and NO_3-N in the biofilters in the mbr-RAS treatment line. The membrane filtration unit lowered the solids concentration in the mbr-RAS resulting in less organic loading, implying reduced heterotrophic competition in the biofilter, and enhanced ammonia conversion in these biofilters. This is in agreement with earlier findings showing that the autotrophic nitrifying biomass can easily be outcompeted by heterotrophic biomass oxidizing available organic compounds (Chen, S. et al., 2006; Guerdat et al., 2011; Michaud et al., 2006).

NH_3-N concentration continued to stay low in the mbr-RAS over time, and the highest reduction compared to the b-RAS treatment line was measured to be 58% for NH_3-N at 30 dph, figure 2a. However, at 22 – 24 dph there was a shift in the concentrations of NO_2-N and NO_3-N, where values for both species decreased in the mbr-RAS compared to the corresponding concentrations in the b-RAS, figure 2b and c, respectively. The highest reduction measured was 77% for NO_2-N and 35% for NO_3-N at 30 dph. The MBR treatment concept was expected to reduce the overall concentration of all nitro-

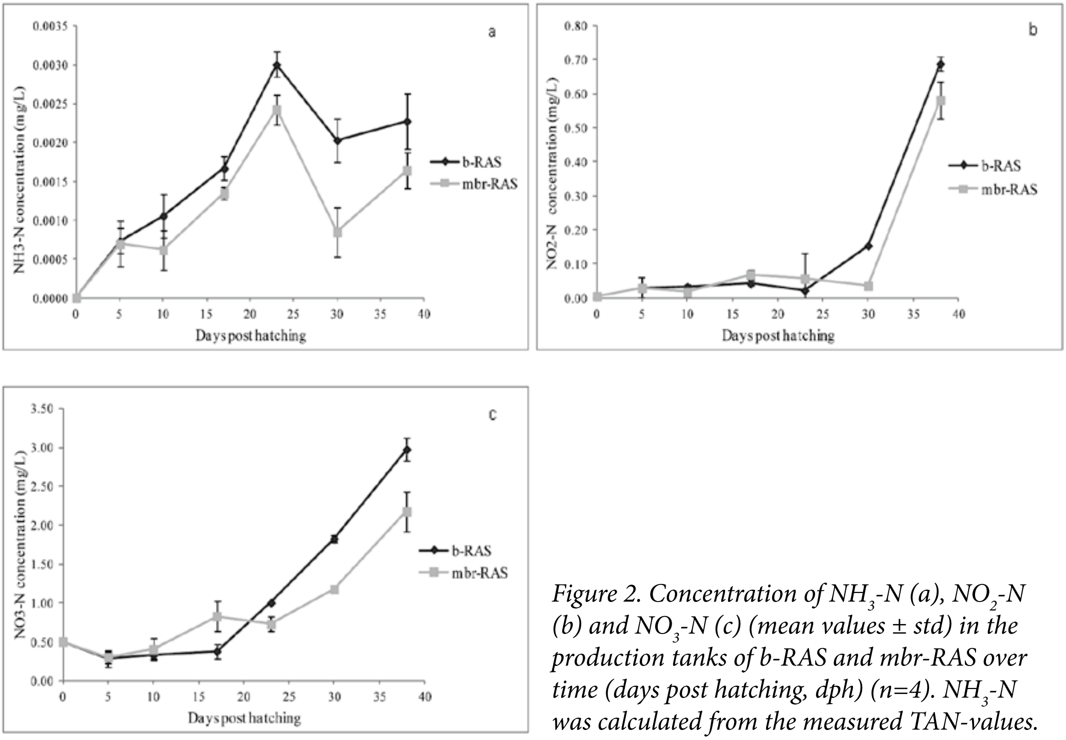

Figure 2. Concentration of NH_3-N (a), NO_2-N (b) and NO_3-N (c) (mean values ± std) in the production tanks of b-RAS and mbr-RAS over time (days post hatching, dph) (n=4). NH_3-N was calculated from the measured TAN-values.

gen compounds in the mbr-RAS due to reduced solids concentration, mineralization and nutrient release. This could explain the observed decrease and lower levels of all N-compounds over time.

Nitrite concentrations during change of feed

As expected, elevated levels of NO_2-N concentration were measured in both treatment systems when changing from Artemia feed (live cells) to dry feed at 33 dph, figure 2b. This response may be due to either an increase in loading rates or reduced activity of the bacteria species oxidizing the nitrification intermediate NO_2-N to NO_3-N indicating that nitrite oxidizing bacteria (NOB) are slower to respond to increased loading rates than ammonia oxidizing bacteria (AOB). The formulated diet in the added dry feed probably has the ability to mineralize much easier in the water compared to live Artemia cells resulting in increased levels of nutrients in the water and thus higher loading rates. Leakage of proteins from formulated diets (dry feed) is extensive already after 5 minutes immersion in seawater (Kvale et al., 2006). NO_2-N concentrations would probably decrease again giving enough time for the bacteria to adapt to this new situation of organic loading.

Membrane operation and performance

Filtering 8.5% of the water flow in the membrane unit led to reduced solids concentration in the mbr-RAS and improved nitrification with ammonia conversion in the biofilter. Furthermore, the solids removal efficiency achieved by the applied membrane operation conditions lowered the content of all N-compounds over time as a result of reduced solids mineralisation and nutrient release. Placing the membrane unit between two biofilter tanks probably resulted in improved nitrification and a higher ammonia conversion in the downstream biofilter receiving the permeate flow. Increasing the filtration rate from 8.5% would most likely result in an even more efficient ammonia conversion in this biofilter, and generally less content of N-compounds in the whole system. Further studies are required to confirm this.

Membrane performance and change in filtration efficiency caused by fouling within the membrane or on the membrane surface can be an operational challenge. Membrane performance was monitored and is reported in a parallel study (Holan, A.B et al., 2013).

Conclusion

To investigate the effect of an advanced system for particle removal on ammonia conversion and nitrogen reduction, a membrane bioreactor (MBR) module was coupled to a recirculating aquaculture system (RAS) and compared to a conventional biofilm RAS. An effect on the water quality was observed in the RAS having a membrane unit filtrating 8.5% of the total RAS flow at any time. Initially, higher levels of the nitrification products nitrite and nitrate, and reduced number of colloidal particles, turbidity, and concentration of ammonia and dissolved organic carbon were observed. The mbr-RAS alternative seemed to create a better environment for nitrification in the biofilters compared to the b-RAS alternative, probably due to a reduced C/N ratio impacting the nitrification rates. Furthermore, the membrane bioreactor alternative reduced the particle concentration in the system and lowered the overall level of N-compounds released through solids mineralization. An increase in NO_2-N concentration was observed in both systems when changing from live feed (*Artemia*) to dry feed at 33 dph. The formulated diet in the added dry feed probably has the ability to mineralize much easier in the water compared to live Artemia cells resulting in increased levels of nutrient release.

By using a small scale experimental setup, the results point in the direction that an enhanced water quality can be achieved when connecting a MBR unit to the recirculating system. This setup seemed to improve the nitrification efficiency, and reduce the content of particulate matter and nitrogen compounds in the water. Inference of results to larger systems is difficult at this stage based on the limited size and scope of the study. Further research is required on the membrane operation and optimization in aquaculture facilities to better understand the effect this may have in the treatment efficiency of the recirculating system and resulting water quality. Future studies would greatly benefit from longer terms experiments and should specifically focus on the performance aspects directly of the biofilter.

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