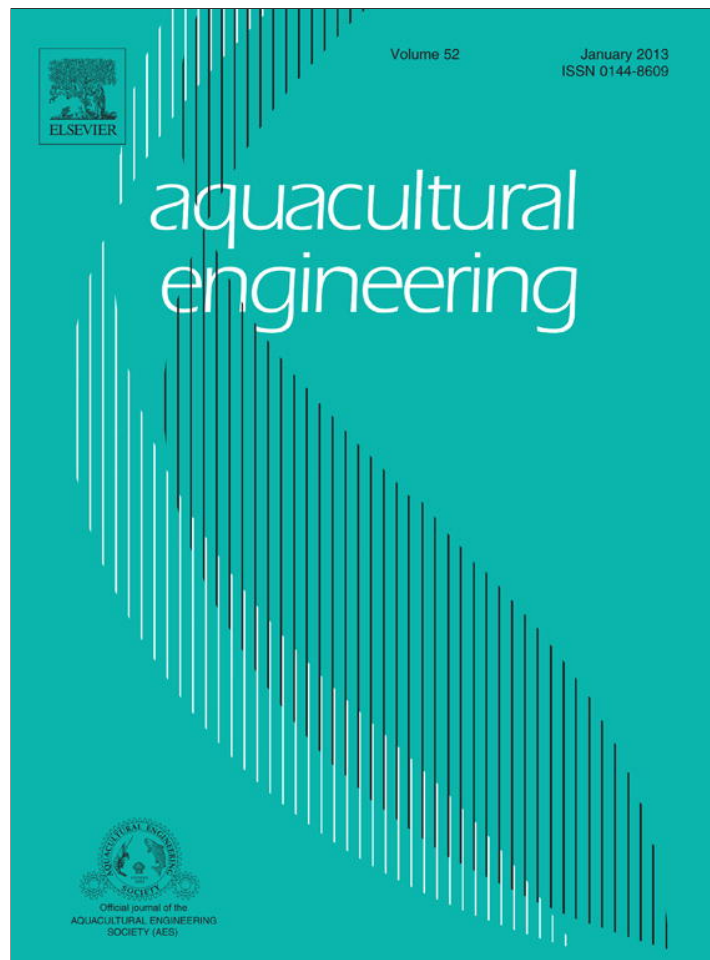


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Performance of sequencing microbead biofilters in a recirculating aquaculture system

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ABSTRACT

Biological filtration, or biofiltration, is the key technology in recirculating aquaculture systems. Sequencing microbead biofilters, in which the media maintains a continuous up-and-down movement, are based on traditional microbead filters but offer superior filtration properties. The performance characteristics of a sequencing microbead biofilter installed in a recirculating aquaculture system for rearing Barcoo perch at $29 \pm 1^\circ\text{C}$ were examined. The total ammonia-nitrogen (TAN) concentrations and the nitrite-nitrogen concentrations during a 52-day culture period were maintained below 1.6 mg/L and 0.9 mg/L. In order to ensure efficient biofiltration, the optimal actual application of hydraulic retention time was determined to be approximately 3–5 min. The water flow produced by the reciprocating motion of the media served to wash away suspended solids, ensuring the occurrence of optimal nitrification processes. Additionally, the reciprocating motion of the media enhanced ammonia treatment efficiency significantly by improving the transport of nutrients and nitrification activity. Compared to a static situation the ammonia removal rate increased by 27% based on the application of up-and-down reciprocating movement. The biofilm on the microbead forms as a compact, complex, and homogeneous structure, consisting of numerous microscopic thin sheets. Additionally, a multitude of pores, interstitial voids, and vertical channels were widely observed to convey obviously advantageous properties in support of fluid passage, thus enhancing mass transfer and ultimately contributing to biofiltration effectiveness. The optimum biofilm thickness for providing efficient biofiltration was determined to be approximately 70 μm for this filter.

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1. Introduction

Recirculating aquaculture systems (RAS) are focused on treatment of nitrogenous wastes, optimization of oxygenation, removal of suspended solids, and control of organic compound accumulation (Brian, 2006). A biological filter is the key technology in the RAS system. Ammonia accumulation in RAS systems is controlled through water exchange and biofilters (Brian, 2006). The system is dependent upon efficient biofilters (nitrifying bacteria) capable of oxidizing the toxic ammonia produced by aquatic organisms into nitrates, which is relatively non-toxic. Microbead biofilters employ a combination of trickling and granular biological filters. Microbead filters use an expanded polystyrene bead that ranges in diameter from 1.0 to 3.0 mm. These biofilters have great potential for use in recirculating aquaculture systems because of their high efficiency, low cost, and stable performance (Timmons et al., 2006).

Conventional microbead biofilters are operated in a down flow configuration. In this configuration, influent water is distributed

over the top of the media bed, and the water subsequently trickles down through the media. Gravity flow then conducts it out of the reactor vessel. Wu et al. (2008) reported a biological filtration method using 2.0 to 3.0 mm diameter beads, each with a volume of 0.2 m³, that had an average total ammonia-nitrogen (TAN) removal rate of 172 g TAN/(m³ day) with influent ammonia-nitrogen levels of 3.0 mg/L. In the Greiner and Timmons (1998) study, the proposed microbead filter possessed nitrification rates ranging from 512 to 2244 g TAN/(m³ day) for influent TAN concentrations between 0.81 and 4.63 mg/L in an intensive recirculating tilapia production facility. Timmons et al. (2006) suggested that microbead filters had a safe nitrify value for designs up to approximately 1200 g TAN/(m³ day) for warm water systems with influent ammonia-nitrogen levels from 2 to 3 mg/L. These filters are simple and reliable in form, but they possess certain limitations due to the thickness of the filter. Excessive filter thickness can cause channeling along the wall and even moderate to severe blockage. The low hydraulic retention time within the bead bed volume will, however, lead to reduced nitrification efficiencies in microbead biofiltration systems (Timmons et al., 2006). If the height of the microbead in the filtration system is too great, or if the width of the chamber is too great, the essentially free flow of water through the center region

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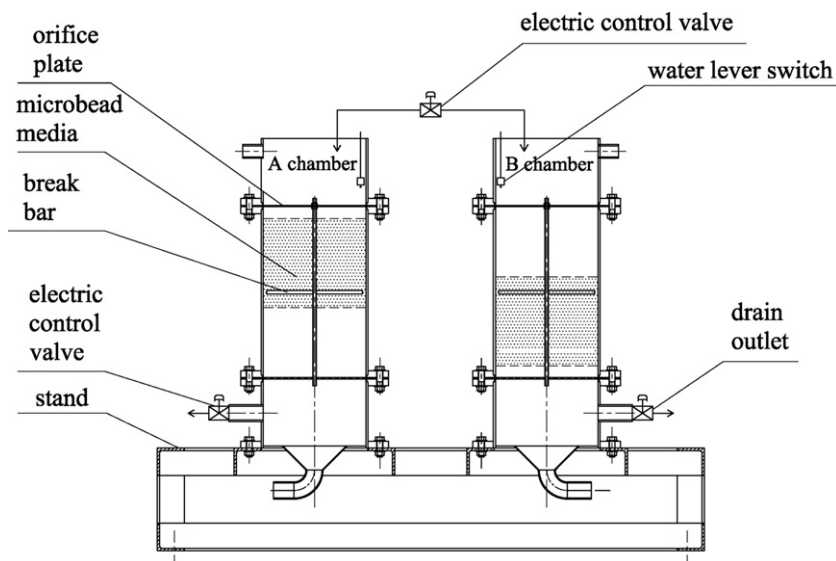


Fig. 1. Design of two-chamber sequencing microbead biofilter. While one chamber was in an inflow state, the other one was drained, proceeded by the electric control valve and water level switch in an alternating fashion.

of the filter tends to initiate water channeling. Water channeling detrimentally decreases the residing time of the reactants passing through the biofilm, thus leading to decreased nitrification activity. The force that prevents water channeling through the media forms the limiting factor for the height as well as diameter of microbead beds. Timmons et al. (2006) considered bead filters to be limited to a depth of approximately 50 cm.

The sequencing microbead biofilter is a successful design modification based on the conventional microbead biofilter concept that was patented by Holder Timmons Engineering, LLC (United States Patent Number US 2007/0056890 "Water filtration system and its use," awarded March 15, 2007). In this modified system, the microbead maintains a continuous up-and-down movement. The breaker bars are positioned in the bead bed volume in order to generate a relative displacement between microbeads, which enable the biofilter obtain the effect of self-cleaning. Microbead biofilter treatment technology is a new recirculating aquaculture water treatment method with potential for use in many commercial systems; however, information on the performance and optimization of sequencing microbead biofiltration in RAS systems is rare. This study seeks to characterize the performance of a sequencing microbead biofilter installed in RAS system, providing a useful case and operation parameter with wide potential applications.

2. Materials and methods

2.1. Equipment

The experimental sequencing microbead biofilter, as shown in Fig. 1, was set up in the Key Laboratory of Fishery Equipment and Engineering, Fishery Machinery and Instrument Research Institute, Chinese Academy of Fishery Sciences. The biofilter was divided into two chambers, designated A-chamber and B-chamber. The water flow through the filtration system was proceed by the electric control valve and water level switch in an alternating fashion, regularly switching between the vessels to enter the A-chamber or B-chamber. Thus, while the A-chamber was in the inflow state, the B-chamber was in the drainage state. Similarly, following the rise of the A-chamber water level and microbead packing layer, the B-chamber water level and bead packing both dropped. When the A-chamber water level reached the level control switch, the switchover occurs by means of the electric control valve associated

with the water level switch. Conversely, while the B-chamber was in an inflow state, the A-chamber was drained. Following the rise of the B-chamber water level and microbead packing layer, the A-chamber water level and bead packing both dropped. The filter achieved the required water level and bead packing in the vessels up-and-down cycle by alternately fill and drain between the two chambers. Each chamber of the experimental filter was 30 cm in diameter and 50 cm height. The filter used expanded polystyrene beads (microbead) approximately 3.0 mm in diameter, with a density of 28 kg/m³ and a specific surface area of 1160 m²/m³. The microbead packing layer was highly filled at 0.26 m, exhibiting a microbead packing volume of 0.037 m³. The filter used a pump to carry water, while the water trickles down the microbead packing due to gravity. Aeration is not required for the filtration system during the experiment.

2.2. Experimental system and conditions

The RAS test system consisting of a 1.3 m³ culture tank, a particle trap, swirl separators, a pump sump, a reuse pump, a sequencing microbead biofilter, and an air diffuser, is shown in Fig. 2. The bulk water was pumped from a sump (water volume, 0.2 m³) to the tested filter after removing solid wastes by the swirl separator, and then returned to culture tank. The total water volume of the system was about 1.6 m³. The pH value was maintained at the range of 6.8–7.2 by dosing with NaHCO₃. The sump was aerated with air through a diffuser so that the dissolved oxygen concentration was maintained at 6.2 ± 0.6 mg/L.

2.3. Experiment design

Generally, this experiment was carried out following three sections:

Section 1: Biofilter startup and operation (with fish).

The experimental system was seeded using 10L of activated sludge from a treatment plant as a source of bacteria. Barcoo perch (*Scortum barcoo*, McCulloch et Waite) was reared in the culture tanks. And the production of large amounts of ammonia could offer nutrient source for nitrifier due to daily fish feeding and fish metabolism. The entire culturing cycle can be divided into the acclimation process, the shock loads process and the stable stage. During the acclimation process, the culturing load was 7 kg fish/m³. When

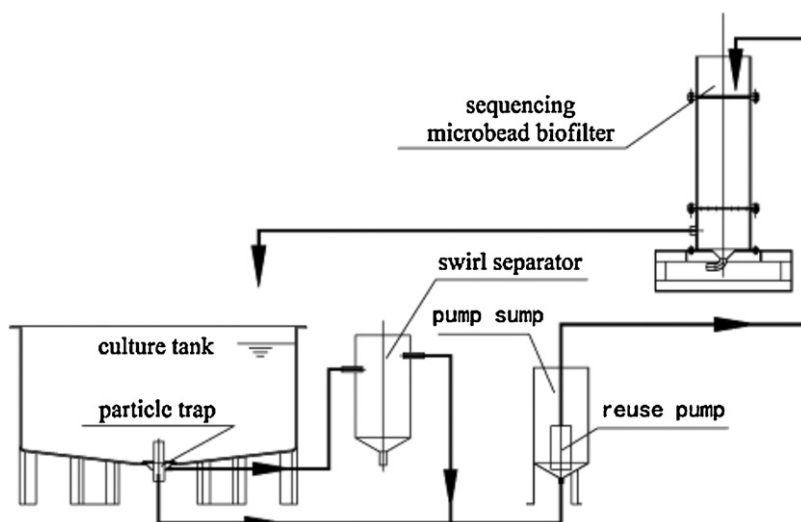


Fig. 2. Diagram of the RAS experiment system.

the ammonia concentration decreased to almost zero, the culturing load was increased to 20 kg fish/m³ in order to test capacity to handle shock loads. Subsequently, the holding density was improved to 38 kg fish/m³ ultimately when the ammonia decreased to almost zero again. Pellet feed with crude protein content of 38% was utilized to feed fish in this experiment. The feeding rates remained at 1% of fish weight, and the temperature remained at 29 ± 1 °C during the test period. Feed was fed to the fishes twice a day, in the morning at 8 am and in the afternoon at 4 pm. The water flow rate in this section was 3 m³/h.

Section 2: Effect of hydraulic retention time (HRT), total suspended solid (TSS), and media motion on TAN removal (without fish).

After about 8 weeks of operation in section 1, a steady-state nitrification was established, as evidenced by a stable TAN concentration for the experimental system. In order to obtain requested water conditions exactly in section 2, a synthetic substrate solution containing ammonia chloride, sodium bicarbonate and other necessary nutrients (Table 1) was continuously fed into the experimental filter instead of fish culture water. The solution was further diluted to TAN concentration of 2.1 mg/L.

(1) HRT

The experiment began with the treatment using the shortest HRT of 1.5 min (for the highest flow rate of 3 m³/h), followed by 2.5, 4, 8, 10, 12 and 15 min (for the lowest flow rate of 0.3 m³/h). Calculate TAN removal rate and removal efficiency in each condition based on the influent and effluent TAN concentration of the filter and research effect of HRT on TAN removal.

Table 1
Composition of substrate nutrients.

Ingredient	Composition (g)
NH ₄ Cl	1377
NaHCO ₃	3500
MgSO ₄ ·7H ₂ O	36
Na ₂ HPO ₄	159
KH ₂ PO ₄	153
FeCl ₃ ·6H ₂ O	5
Water	701

The solution was further diluted for the requirement of different experimental conditions.

(2) TSS

The TSS concentration in the experimental system ranged from 6 to 33.2 mg/L. Calculate TAN removal rate in each condition and observes effect of TSS on the experimental filter.

(3) Media motion

The reciprocating motion was suspended for six days. Then resume movement of the media and compare the ammonia removal rate in two operation conditions.

Section 3: Biofilm structure and thickness.

The synthetic substrate solution was further diluted to TAN concentration of 2, 6, 8, 10 mg/L. These water of different TAN concentration was continuously pumped through the experimental filter at a given condition of HRT = 5 min. When the TAN feeding rate was changed, it took 7 days for the filter to acclimate the change of environment. Samples of microbead at each condition collected and observed by scanning electron microscopy (SEM).

2.4. Sampling methodology and water quality analysis

Section 1: Daily samples were taken from the sump of the experimental system during the operation of the filter in a row at 10 am, 2 h after feeding.

Section 2: Water samples were collected from both the inlet and outlet of the biofilter units associated with the system.

Samples were analyzed for TAN (total ammonia nitrogen), NO₂-N (nitrite-nitrogen), and TSS (total suspended solids) using the standard methods of 4500-NH₃-C, 4500-NO₂-B and 2540-TSS-E, respectively (APHA, 2001). Water temperature, pH and DO (dissolved oxygen) were assessed by YSI 556MPS Multi-Parameter Water Quality Instrument (YSI, USA).

2.5. Data analysis

The efficiency of the biofilters in the removal of nitrogenous waste was determined by using the estimated waste loading and removal rates as follows:

$$\text{Waste removal rate (g/m}^3 \text{ per day)} = \frac{(C_i - C_e)Q}{Vol} \tag{1}$$

$$\text{Removal efficiency (\%)} = \frac{C_i - C_e}{C_i} \times 100\% \tag{2}$$

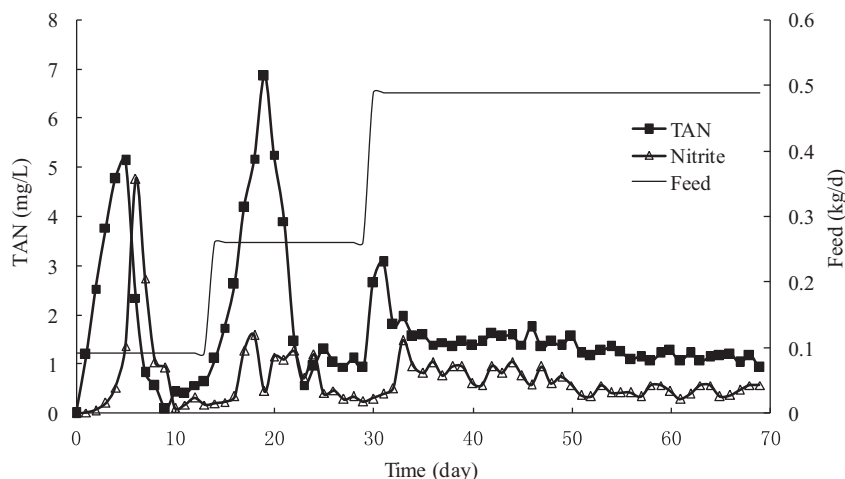


Fig. 3. Performance of a sequencing microbead biofilter. The feed was increased from 0.09 to 0.26, finally 0.49 kg/day according to the change of TAN and nitrite.

where C_i is the influent waste concentration (g/m^3), C_e is the effluent waste concentration (g/m^3), Q is the water flow rate (m^3/day) and Vol is media volume (m^3). The means of analysis for water quality parameters and percent waste removal in the biofilters was constructed using Microsoft Excel 2007 software.

3. Results and discussion

3.1. Biofilter startup and operation

The biofilter was able to establish nitrification functions ranging from 10 to 20 days in freshwater systems (Carmignani and Bennett, 1977; Yang and Yan, 1988). Based on the experimental results shown in Fig. 3, which details the sequencing microbead biofilter startup and operation in this experiment, the cultivation of nitrification function required 8 days, spending less time compared with previous studies of 11 days (Carmignani and Bennett, 1977), 15 days (Yang and Yan, 1988) and 17 days (Luo et al., 2010). The shorter activation period was likely due to the operation of the experimental biofilter with a low load at startup. These findings are consistent with the results reported demonstrating putting appropriate load fish directly and feed lowly was the best method to establish the nitrification function of the biofilter (Luo et al., 2006). This research provided another experimental evidence for the theory that low fish stocking density (less than $10 \text{ kg fish}/\text{m}^3$ in this study) is effective in reducing activation time of filters.

After formation of nitrification function, the fish load was improved dramatically, followed by a sharp increase in the TAN concentration and minimal fluctuations in nitrite-nitrogen concentrations. Because the growth rate of ammonia oxidizing bacteria is far slower than that of nitrate oxidizing bacteria, the number of initial bacterial population was not stable enough to reach an environmental equilibrium (Carmignani and Bennett, 1977). Feed and fish load on the system was increased, the TAN concentration reached maximum in another 4 days, as shown in Fig. 3. Afterwards, the TAN concentration rapidly decreased to $0.6 \text{ mg}/\text{L}$, and a balance was achieved after 8 days. Additional increases the fish load to $38 \text{ kg fish}/\text{m}^3$ did not cause obvious changes in water quality, indicating a gradually stabilization of the filter over time. The TAN concentrations and the nitrite-nitrogen concentrations during the 52-day stable-stage period were maintained below $1.6 \text{ mg}/\text{L}$ and $0.9 \text{ mg}/\text{L}$.

3.2. Effect of hydraulic retention time (HRT) on TAN removal

The effect of HRT on TAN removal at the influent TAN concentration of $2.1 \text{ mg}/\text{L}$ in this experiment is shown in Fig. 4. The TAN

removal efficiency and the removal rate of the biofilter are affected by HRT. The ammonia-nitrogen removal rate was in direct proportion to HRT, while the TAN removal efficiency was in inverse proportion to HRT. When the HRT was greater than 10 min, the TAN removal efficiency of the biofilter was higher than 60%; however, the TAN removal rate of the biofilter remained under $400 \text{ g}/(\text{m}^3 \text{ day})$. Conversely, when the HRT was 2 min, the TAN removal efficiency of the biofilter reduced to approximately 20%. The TAN removal rate in the biofilter was greater than $600 \text{ g}/(\text{m}^3 \text{ day})$. As the HRT increased in the biofilter, the single-pass TAN removal efficiency increased correspondingly. Unfortunately, this benefit is coupled with a decline in the actual handling ability, as demonstrated in Fig. 4. The intersection of the two curves at approximately 5 min in Fig. 4. Taking into account the TAN removal rate in practical implication, efficient biofiltration can be ensured when the HRT is set between 3 and 5 min. This not only ensures optimal biofilter treatment effects, but also enables the filter to maintain a high speed as the microbead packing passes through the breaker bar up-and-down. The relative motion of the microbeads causes them to exert friction and extrusion forces with each other. Aging biofilms may be dislodged in a timely fashion, ensuring maintenance of the good condition of the biofilm (Hu et al., 2005), resulting in the notable achievement of biofilter self-cleaning and high efficiency.

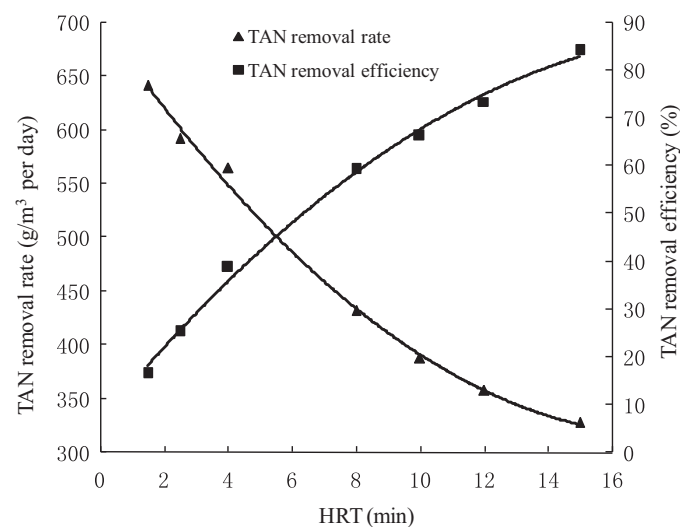


Fig. 4. Impact of hydraulic retention time on TAN removal at influent TAN concentration of $2.1 \text{ mg}/\text{L}$.

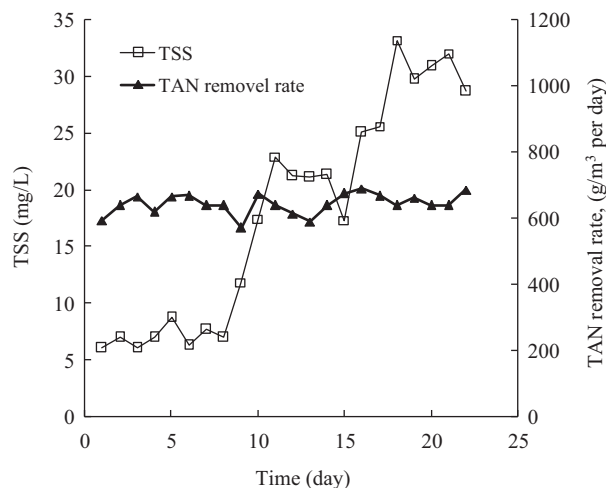


Fig. 5. Impact of TSS on TAN removal. The synthetic solution was diluted to TAN concentration of 2.1 mg/L.

3.3. Effect of total suspended solid (TSS) on ammonia removal

Water with high TSS levels may easily cover the surface of the biofilm, influencing biological oxidation processes, thus leading to a sharp drop in nitrification (Xia et al., 2003). In order to test the impact of TSS levels on the tested filter, an experiment investigating different TAN removal rate with changing TSS concentration was performed, as shown in Fig. 5. When the influent TSS levels were in ranges between 6 and 33.2 mg/L, the removal rate of ammonia remained between 593 and 685 g/(m³ day). The change observed with TSS was very small, indicating that the fluctuations of influent TSS concentration generated no perceptible effect on the nitrification process. As rise and fall of the water level in the filter the filter media's high-speed reciprocating movement intensified the friction between the filter beads. This action washed these adhered solids away and settled at the bottom of the filter, ensuring the process of nitrification. The result of this experiment indicated that it could overcome fixed bed biofilters' disadvantage that suspended solid weakened their nitrification efficiency greatly.

3.4. Effect of media motion on nitrification efficiency

The mass transfer of the substrate and nutrients across the biofilm is an essential factor in effective nitrification efficiency, a factor often proven to be the limiting step in such systems. The mass flow of nutrients from the liquid phase to the biofilm determines the ammonia removal rate possible for a biological filter. The turbulence in the biofilter affects the water boundary layer thickness, thus affecting the resistance of the substrate transfer process from the bulk to the biofilm. Therefore, the turbulence in the biofilter has a significant impact on the mass transfer process and nitrification efficiency. Stoodley et al. (1997) researched the relationship between the mass transfer coefficient and flow rate in heterotrophic biofilms using microelectrodes and confocal scanning electron microscopy. In the study, the variation of average mass flow rate was found to lead to nonuniformity of mass transfer. Zhu and Chen (2001) studied the effect of the Reynolds number on ammonia removal efficiency. These results showed that the flow Reynolds number had a significant impact on the nitrification rate in theory as well as practice, demonstrating that the hydraulics on the surface of a biofilm were crucial factors affecting ammonia removal efficiency. Kugaprasatham et al. (1991) studied the influence of water hydraulics on the nitrification of cylindrical reactors in low ammonia environments. When alterations were made

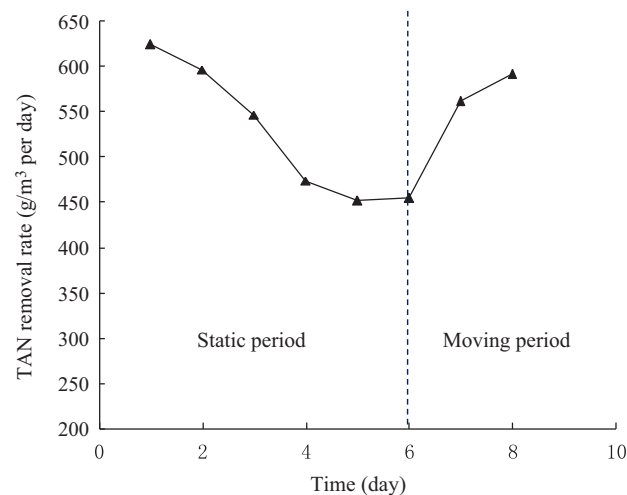


Fig. 6. Impact of media movement on TAN removal.

to the turbulence over the course of several days, the transfer of filamentous-type biofilm proved highly efficient in elevated turbulence, while colony-type biofilm showed dramatically reduced transfer in low turbulence conditions. Ling and Chen (2005) also reported that higher levels of turbulence led to increased efficiency in biofiltration systems.

One of the typical unique characteristics of the sequencing microbead biofilter is that its media is maintained in constant reciprocating motion. The relationship between the ammonia removal rate and such media layer movement was investigated in the current system, with results revealing that the movement enhanced the treatment performance dramatically (Fig. 6). Six days later, when the reciprocating motion was suspended, the ammonia removal efficiency exhibited a gradual decline of 27%. Upon resumed movement of the media, the biological filter ammonia removal efficiency increased rapidly until it reached levels observed prior to the two-day motion suspension. This effect can be attributed to the media reciprocating movement that cuts the flow, thus greatly improving the hydraulics on the surface of the media and controlling the biofilm thickness and biomass age. Also, increasing the turbulence in the biofilter improves the mass transfer efficiency between the three elements of biological treatment: microorganisms, dissolved oxygen, and contaminants. Therefore, biofilm activity and nitrification efficiency increase correspondingly.

3.5. Biofilm structure and thickness

The surface of a microbead in the experimentally tested biofilter in steady-state operation was observed by scanning electron microscopy (SEM) (Fig. 7a and b). Gjaltema et al. (1994) reported that the structures of biofilms were affected by the type of microorganisms, the surface of the media, the liquid flow, the type of reactor, biofilm loss, and other varied factors. de Beer et al. (1994) and Massol-Deya et al. (1995) pointed out that there were cavities, voids, and channels in variety of groups of symbiotic biofilms (aerobic and anaerobic membranes). As seen in Fig. 7, biofilms are seemingly uniform and dense; however, further examination by magnification reveals that the structure of biofilms is actually very complex, consisting of many thin layers of uniform thickness. Cavities, voids, and channels were all observed in the research of de Beer et al. (1994) and Massol-Deya et al. (1995) as well as the current study. Yang and Lewandowski (1995) presented that increased partial mass transfer efficiency of these

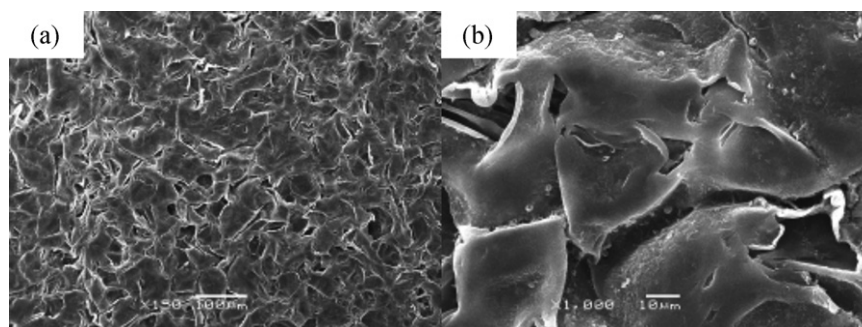


Fig. 7. SEM of the nitrifying biofilm: (a) 150 \times and (b) 1000 \times .

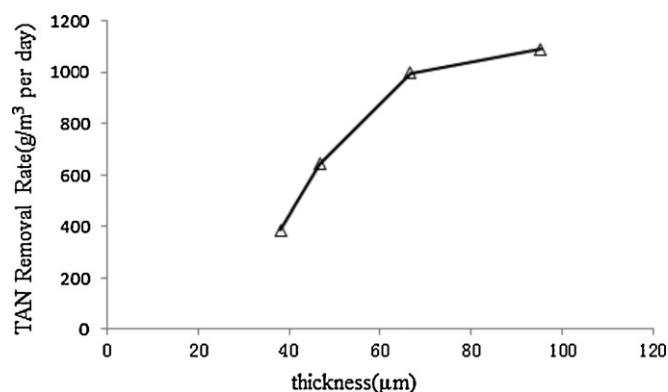


Fig. 8. Relationship between TAN removal rate and biofilm thickness.

structures based on principles of fluid convection allowed for an increase in biofilm activity and therefore nitrification rate elevation.

Many researchers have proposed the hypothesis of “the best suitable biofilm thickness” (Wang and Zhou, 1994; Yoda et al., 1987). Though variable quantitatively, this principle takes into account that filter performance is much more efficient when biofilm thickness is suitable (Andrews and Przedziecki, 1986; Wang and Shen, 1991; Wang and Zhou, 1994; Yoda et al., 1987). The optimal thickness of biofilms is impacted by many factors, such as the characteristics of the media, substrate concentration, substrate type, and volume load. Hydraulic conditions in the biofilter can also affect the formation of the biofilm. Kugaprasatham et al. (1992) suggested that turbulence of the liquid will be the primary factor as it increases the density of the biofilm at non-limiting substrate conditions. For media with variant diameters, the optimal biofilm thickness can be calculated and experimentally tested (Andrews and Przedziecki, 1986). For the same media type, however, the optimal film thickness varied with substrate concentration. Furthermore, for different substrates, the optimal thickness also varied (Buffière et al., 1995).

Based on the parameters observed in previous research, the current study was designed to investigate the relationship between biofilm thickness on the media surface and ammonia removal rate, as shown in Fig. 8. The ammonia removal rate increased almost linearly with increasing biofilm thickness for biofilms with thicknesses ranging from 38.2 to 66.7 μm . For biofilm thicknesses above 66.7 μm , however, the trend plateaued, indicating only an imperceptibly slight increase at further increased thicknesses. Thus, conclusions can be drawn that the optimal biofilm thickness for the experimental biofilter was approximately 70 μm , a value concurrent with the ranges identified in previous research studies.

4. Conclusions

To investigate ammonia removal performance of sequencing microbead biofilter a pilot scale recirculating aquaculture system was developed. At the startup period of the filter, Barcoo perch was reared in the culture tanks. When the water temperature was 29 ± 1.2 $^{\circ}\text{C}$ and the culturing load was $7 \text{ kg fish}/\text{m}^3$, the establishment of nitrification function of the experimental filter was about 8 days, spending less time compared with previous studies. The shorter activation period results from the low culturing load at startup. Then, the culturing load was gradually increased to $38 \text{ kg fish}/\text{m}^3$. In the stable-stage of 52 day, TAN and the nitrite-nitrogen concentrations were maintained below 1.6 mg/L and 0.9 mg/L.

After the biofilter startup and operation experiment, the filter started receiving artificial solution instead. For synthetic wastewater, effect of hydraulic retention time (HRT), total suspended solid (TSS), and media motion on TAN removal were investigated. The results showed that: (1) the TAN removal rate was in direct proportion to HRT, while the TAN removal efficiency was in inverse proportion to HRT. The optimal HRT suggested was 3–5 min in the actual application. (2) The fluctuations of influent TSS concentration generated little effect on the nitrification process, indicating that it could overcome fixed bed biofilters' disadvantage that suspended solid weakened their nitrification efficiency greatly. (3) The reciprocating motion of the media increased the ammonia treatment efficiency significantly by improving the transport of nutrients in biofilm and nitrification activity. Compared to values observed under static conditions, the ammonia removal rate increased by 27%.

Based on the results of SEM, biofilm microbeads form a compact, complex, and homogeneous structure that consists of numerous thin sheets, visible under magnification. Additionally, pores, interstitial voids, and vertical channels are characteristic of the microbeads, playing a part in their distinct mechanical, thermal, and chemical characteristics. These structures convey obvious advantages in that they allow fluids to pass unimpeded, thus enhancing mass transfer. For the examined biofilter, the optimal biofilm thickness was approximately 70 μm , a reasonable approximation for similar biofilm systems operated under similar conditions.

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