Application of Membrane Bioreactor with Sponge Media in Aquaculture Wastewater Treatment

Ratboren Chan, Chart Chiemchaisri* and Wilai Chiemchaisri

ABSTRACT

A single-stage aerobic membrane bioreactor (MBR) has been developed as an efficient and compact system for aquaculture wastewater treatment. In this study, the role of sponge media in an MBR treating synthetic aquaculture wastewater was investigated. The MBR was operated at a hydraulic retention time of 4 h and its treatment performance during the operation with and without sponge media was compared. During 120 days of operation, there was no significant effect of sponge media on the organic, ammonium or organic nitrogen removal observed in the MBR. High organic removal was achieved, i.e. 98.3 % for biochemical oxygen demand (BOD) and 85.2 % for chemical oxygen demand (COD), whereas ammonium and total Kjeldahl nitrogen (TKN) removal was 98.5 % and 88.3 %, respectively. Nevertheless, the integration of attached biomass on sponge media helped by significantly improving total nitrogen (TN) removal from 38.7 % to 53.4 % through enhanced denitrification activity of the MBR biomass, even though it was operated under aerobic conditions. Batch experiments confirmed higher denitrification activity (0.58 $mg \cdot g^{-1} \cdot h^{-1}$) of attached biomass than suspended biomass (0.23 $mg \cdot g^{-1} \cdot h^{-1}$). Higher first-order nitrification rate was observed in suspended biomass (0.21 h^{-1}) than in attached biomass (0.12 h^{-1}), whereas denitrification rate was higher in attached biomass (0.14 h^{-1}) than in suspended biomass (0.06 h^{-1}). The MBR with sponge media can be considered as an alternative treatment system for controlling water pollution in a recirculating aquaculture system where available land area for wastewater treatment is limited.

Keywords: Aquaculture wastewater, MBR, Nitrogen removal, Polyurethane sponge

INTRODUCTION

Wastewater generated from aquaculture farms contains risk hazards in terms of pollutants, including organics, nutrients, and toxic compounds (Konnerup *et al*., 2011). Wastewater directly discharged from aquaculture farms to the environment without proper treatment can create adverse impacts to human health and ecosystems (Cao *et al*., 2007). Therefore, an effective wastewater treatment is necessary to achieve sustainable operation of a recirculating aquaculture system (RAS) or to minimize environmental risk from discharging aquaculture wastewater into the natural environment. Generally, the major pollutants contained in aquaculture wastewater are suspended solids, dissolved organic and nitrogen compounds leftover from aquaculture feed. Regarding these compounds, maintaining low concentrations of residual nitrogen in aquaculture water is a key requirement for the RAS system due to the potential detrimental effects of ammonia and nitrate on fish growth and metabolic activities (Zou *et al*., 2018).

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Several methods ranging from conventional to advanced techniques have been applied to the treatment of aquaculture wastewater. Physical separation of solids is commonly used for reducing pollution from fish farm effluent. Nevertheless, it is ineffective in eliminating other pollutants in dissolved forms. Meanwhile, intensive treatment systems are less frequently used (Kõiv *et al*., 2016). Simple treatment systems associated with low operation cost such as wetlands or biological filter systems have been proposed (Bôto *et al*., 2016; Crab *et al*., 2007). Moreover, the combination of conventional and biological treatment to enhance nutrient removal, such as trickling filter or fluidized bed reactor followed by air stripping and oxygenation have also been applied (Chiam and Sarbatly, 2011). For nitrogen removal, nitrifying trickling filters (Eding *et al*., 2006) and media, or slow sand filtration (Lindholm-Lehto *et al*., 2020) have been utilized. Nevertheless, those treatment techniques require large installation area and multiple treatment steps. Recently, membrane bioreactor (MBR) technology, an integration of activated sludge with membrane filtration, has been proposed as a compact and efficient treatment system for aquaculture wastewater (Thanh *et al*., 2013). The advantages of MBR technology are improved performance from conventional treatment systems, smaller footprint area and less excess sludge production due to the long sludge retention time maintained in the system (Iorhemen *et al*., 2016). However, one of the challenges in removing nitrogenous compounds from wastewater in MBR is to achieve a high degree of nitrification during a short treatment time due to the slow growth rates of autotrophic nitrifying microorganisms. Moreover, the system would require multi-step treatment to achieve both nitrification and denitrification reactions to complete nitrogen removal from wastewater in the form of nitrogen gas (Chiemchaisri and Liamsangoun, 2004). Another challenge in achieving complete nitrogen removal in a single-stage biological reactor is due to carbon competition between ordinary heterotrophic and denitrifying microorganisms (Yin *et al*., 2015). To overcome those limitations, integration of attached biomass into a single-stage MBR was examined by Yang *et al*. (2009) in an attempt to enhance nitrogen removal. Effective nitrogen removal in a moving-bed MBR was achieved due to the promotion of growth of a slow-growing microorganism (Yang *et al*., 2010). To facilitate biomass attachment on media, several types of carriers have been employed, e.g. chitosan (Gentili *et al*., 2006), ceramics (Dong *et al*., 2011), polyurethane foam and polymer (Chu and Wang, 2011) and fiber threads (Jin *et al*., 2012). Among them, polyurethane (PU) sponge is considered highly effective for utilization in MBR because it has high porosity to serve as a medium for microbial growth, it improves the efficiency of biodegradation, it enhances the nitrification process, and it can limit oxygen availability inside the sponge for denitrification (Guo *et al*., 2010; Liu *et al*., 2010). Khan *et al*. (2011) compared the treatment efficiencies of a conventional MBR utilizing suspended biomass only with a sponge media-incorporated MBR and found that nitrogen removal was enhanced in the latter MBR type. Furthermore, sponge media also helped reduce membrane fouling, thus extending effective operation period of the membrane filter (Ngo *et al*., 2008; Thanh *et al*., 2013).

Positive impacts of attached biomass on nitrogen removal in biological treatment systems including MBR have been reported. Lim *et al*. (2012) explained the nitrogen removal in a moving-bed sequencing batch reactor operated with intermittent aeration. In that system, suspended growth biomass played a major role in ammonium nitrogen oxidation, whereas stored carbon substrate in the biomass located inside the acclimated PU foam cubes was responsible for facilitating the denitrification process. Nguyen *et al*. (2016) reported that sponge-MBR achieved 9-16 % more removal of total nitrogen than MBR due to the majority of total biomass being entrapped in the sponges, which enhanced simultaneous nitrification-denitrification. Chu and Wang (2011) reported that biodegradable polymer in a moving-bed reactor could serve as effective substrate, providing reducing power for denitrification. Nevertheless, the role of attached biomass in nitrogen removal in single-stage aerobic MBRs has been less explored. Moreover, kinetic rates of nitrogen removal through nitrification and denitrification reactions carried out by suspended and attached biomass in single-stage aerobic reactors have not been reported. Therefore, this research aimed to investigate the effect of polyurethane (PU) sponge media in MBR on the enhancement of nitrogen removal from aquaculture farm effluent. The kinetic rates of nitrogen removal by suspended and attached biomass on PU sponge media were also derived from batch experiments.

MATERIALS AND METHODS

MBR setup and operation

A laboratory-scale MBR (Figure 1) made from acrylic material with 5 L working volume (15×10×45 cm) was used in this study. Three hollow-fiber membrane modules (Sterapore SADF™, PVDF material, 0.4 μm pore size, 0.105 m^2 surface area each) were installed in the MBR. Air was supplied through diffusers to maintain dissolved oxygen (DO) at 5-6 $mg{\cdot}L^{-1}$ as well as provide scouring of the membrane surface to reduce fouling. Synthetic aquaculture wastewater was fed into the MBR using a peristaltic pump controlled by a level sensor. Meanwhile, the permeate was pumped from the membrane modules controlled by a suction pump at a constant rate of 30 $L{\cdot}day^{-1}$, thus yielding average hydraulic retention time (HRT) of 4 h in the MBR. The MBR was operated as a single run of an experiment under two different conditions: 1st) with only suspended biomass at 5 $g{\cdot}L^{-1}$ concentration (day 1-40) and 2nd) with suspended and attached biomass on sponge media (day 41-120) by addition of sponge cubes at 10 % of reactor volume. The sponge cubes were made of polyurethane material with 1×1×1 cm size (Figure 2).

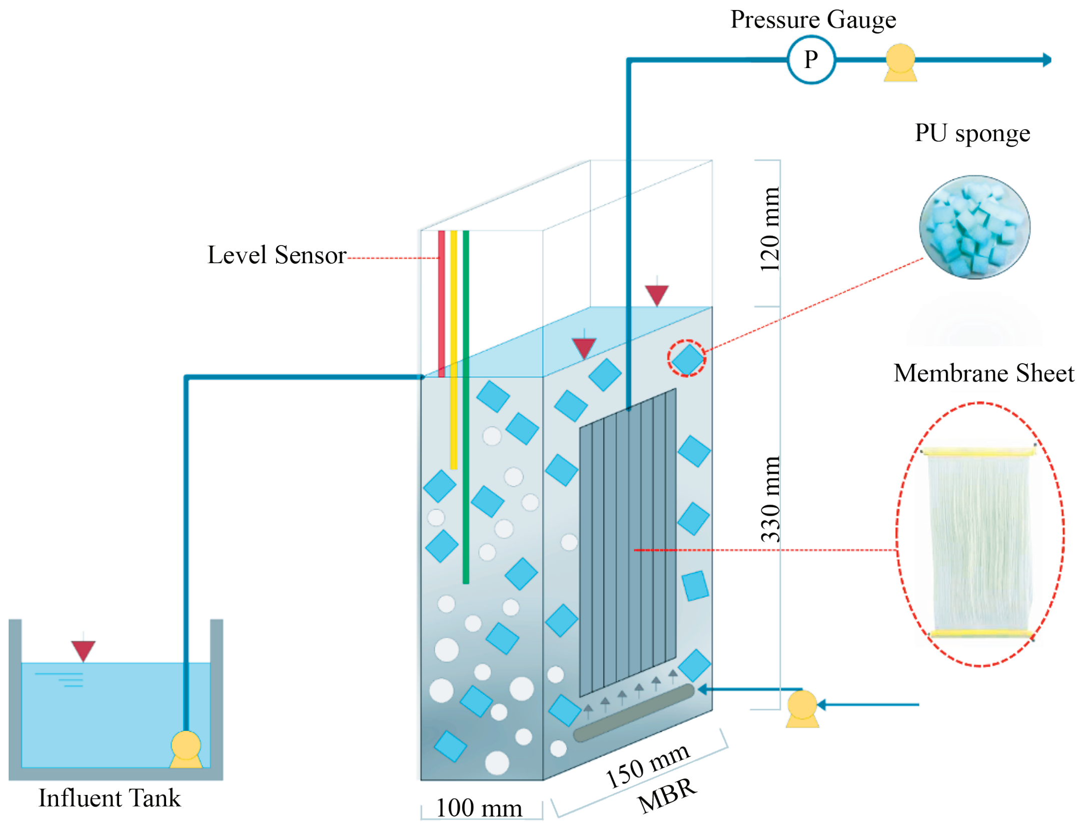

Figure 1. Schematic of membrane bioreactor (MBR).

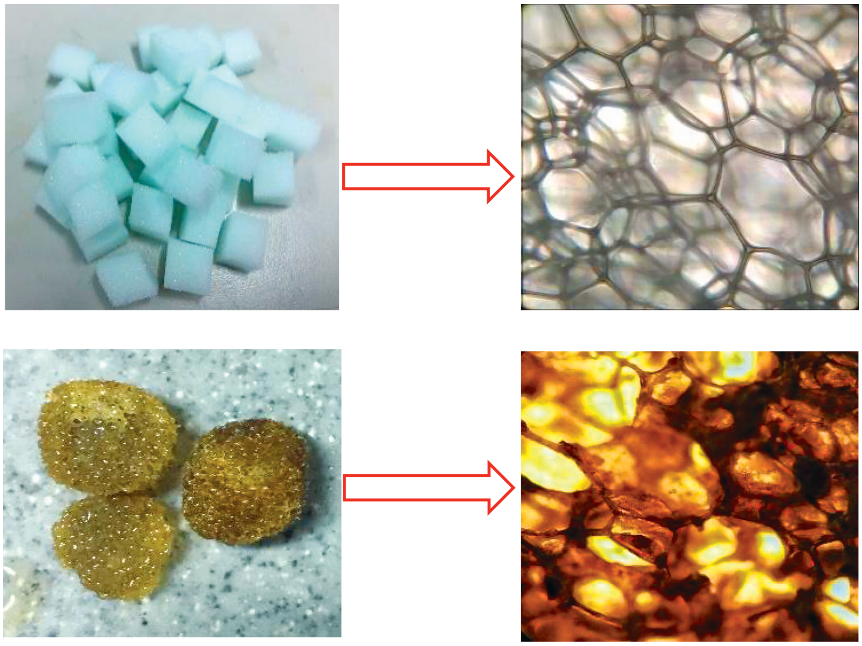

Figure 2. Photographs and micrographs (100×) of PU sponges before and after use.

Feed wastewater and water quality analysis

Simulated aquaculture wastewater was prepared by mixing fish feed (30-50 % protein content) into fishpond water having initial ammonium concentration of 3-4 mg·L^{-1} without additional salinity supplement. The main components of the fish feed were protein, fat, crude fiber, nitrogen-free fextract, ash and moisture. Fish feed (60 g) was mixed well with 100 L of fishpond water for 24 h to allow for complete homogenization. Then, the prepared water was screened through a coarse filter (5 µm cartridge filter) to remove remaining suspended solids before feeding it to the MBR. The characteristics of synthetic wastewater are shown in Table 1.

Table 1. Characteristics of synthetic wastewater used under both operating conditions.

Parameters	**1st condition (suspended biomass only)**	**2nd condition (suspended and attached biomass)**
BOD (mg·L^{-1})	174±29	139±28
COD (mg·L^{-1})	235±38	226±55
TOC (mg·L^{-1})	62.5±3.9	58.4±6.7
SS (mg·L^{-1})	49.5±23.7	51.2±16.9
NH_4^+-N (mg·L^{-1})	4.9±0.6	5.2± 0.1
TKN (mg·L^{-1})	6.5±0.9	7.1±1.1
NO_2^--N (mg·L^{-1})	0.1±0.05	0.1±0.05
NO_3^--N (mg·L^{-1})	0.01±0.0	0.1±0.01
TN (mg·L^{-1})	6.51±0.9	7.2±1.1

All liquid samples (influent and effluent) were collected twice a week. Physicochemical characteristics such as suspended solids (SS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and total Kjeldahl nitrogen (TKN) were analyzed in triplicate following Standard Methods for the Examination Water and Wastewater (APHA, 2012). Total nitrogen (TN) was calculated from the summation of NH_4^+, NO_2^-, NO_3^-, and organic nitrogen while the organic nitrogen was determined from the difference between TKN and NH_4^+. In addition, total organic carbon (TOC) was determined by the TOC analyzer (Shimadzu-VCSH, 511045 series).

The suspended biomass in the system was also regularly analyzed in terms of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). The attached biomass on sponge media was collected by manually squeezing it into a known volume of distilled water. The resulting solution was then analyzed for SS and volatile suspended solid (VSS) concentrations, and the attached biomass on the sponge media was then calculated in terms of solid mass (VS basis) per piece of sponge.

Nitrogen removal and related microbial activity

To verify nitrogen removal mechanisms in the MBR, mass balance of total nitrogen was determined following Equation 1.

$$TN_{inf} = TN_{assimilate} + TN_{denitrification} + TN_{eff} \quad \text{Equation 1}$$

Where TN_{inf}, TN_{eff} are total nitrogen mass in the influent and effluent, respectively. $TN_{assimilate}$ is total nitrogen mass assimilated into biomass which was estimated from the nitrogen content in biomass at 12 % of MLVSS (weight basis) (Ammary, 2004) and $TN_{denitrification}$ is the total nitrogen mass removed through denitrification. The determination of nitrogen mass balance was carried out during one month during continuous operation of the system.

Additionally, batch experiment tests were carried out to determine nitrification and denitrification capacities of suspended, attached, and combined biomass. The experiments were performed in sterilized bottles with 250 mL working volume. The suspended and attached biomass for batch experiments were prepared at the same proportions as those observed in the MBR. The suspended biomass concentration was set at 1.5 $g{\cdot}L^{-1}$, whereas attached sludge on 10 sponge cubes with an average of 1.3 $g{\cdot}g^{-1}$ sponge of attached biomass was employed. Aeration was continuously supplied to maintain DO at 5-6 $mg{\cdot}L^{-1}$. The experiments were performed over 4 h, during which samplings were performed on an hourly basis. NH_4Cl (153 $mg{\cdot}L^{-1}$) was used as substrate in the nitrification test whereas KNO_3 (400 $mg{\cdot}L^{-1}$) and glucose (187.5 $mg{\cdot}L^{-1}$) were used in the denitrification test. All experiments were carried out in triplicate.

The specific nitrification rate (NR) and denitrification rate (DR) of biomass responsible for biological nitrogen removal in the MBR were then evaluated using Equations 2 and 3 and expressed as $mg{\cdot}gVS^{-1}{\cdot}h^{-1}$, where volatile solids (VS) were determined from total biomass examined, i.e., suspended or attached biomass or both.

$$NR = \frac{[(NH_4^+)_{inf} - (NH_4^+)_{eff}]}{VS \times t} \quad \text{Equation 2}$$

$$DR = \frac{[(NH_4^+)_{inf} - (NH_4^+)_{eff} + (NO_2^- + NO_3^-)_{inf} - (NO_2^- + NO_3^-)_{eff}]}{VS \times t} \quad \text{Equation 3}$$

Where $(NH_4^+, NO_2^-, NO_3^-)_{inf,\ eff}$ are ammonium, nitrite and nitrate mass in the influent and effluent during the MBR operation period, respectively. VS is total volatile solid mass and t is the number of operation hours of MBR during which NR and DR are determined.

The reaction rate constants for nitrification and denitrification in the batch experiments were also derived following the first-order kinetic expression as follows:

$$\frac{dS}{dt} = -kS \qquad \text{Equation 4}$$

Where dS/dt is the substrate (NH_4^+ or NO_3^-) removal rate ($mg{\cdot}L^{-1}{\cdot}h^{-1}$), k is the first-order rate constant (h^{-1}) and S is the substrate concentration in the reactor ($mg{\cdot}L^{-1}$).

Statistical analysis

The pollutant removal efficiencies under two operating conditions were compared using t-test (two-sample, assuming equal variances). The test results were considered significantly different at $p<0.05$. Microsoft Excel version 2010 was used for the statistical analysis of the experimental data.

RESULTS AND DISCUSSION

Biomass presence in the MBR

The MBR was initially operated to develop the biomass in the system. During this period, the biomass (measured in terms of MLSS) was allowed to increase steadily until a stable condition was reached. It was found that MLSS increased to a maximum concentration of 8.5 $g{\cdot}L^{-1}$, yielding a biomass production of 0.25 g $MLSS{\cdot}g^{-1}$ BOD removed. To maintain a constant biomass concentration of 5 $g{\cdot}L^{-1}$ during the 1st condition (day 1-40), about 150-200 $mL{\cdot}day^{-1}$ of mixed liquor sludge was withdrawn from the reactor. Under this operation, the calculated solid retention time in the system was 30 days. In the 2nd condition (day 41-120), sponge media was added to the MBR so that the biomass was present in two different forms, i.e., suspended biomass and attached biomass on sponge media. The control of suspended biomass concentration through sludge withdrawal yielded solid retention time of 80 days under this condition. A decrease in suspended biomass (MLSS) from 5 to 1.3 $g{\cdot}L^{-1}$ was observed during day 41-90 under this operating condition (Figure 3); it resulted from the attachment of suspended biomass onto the sponge media until the sponges were saturated. Afterwards, the biomass was developed further, mainly in suspended form, during day 90-120, when an average MLSS concentration of 4.5 $g{\cdot}L^{-1}$ was observed. During steady operation, the attached biomass on sponge media was found to be 1.5 $g{\cdot}g^{-1}$ sponge. The total biomass in the MBR operated under this condition was determined as 22.50 g (58.5 %) and 15.95 g (41.5 %) in suspended and attached forms, respectively (Figure 4). It is noteworthy that the operating MLSS concentrations in the MBR of this study were much lower than has been reported as a critical level for membrane fouling, e.g., 10 $g{\cdot}L^{-1}$ (Prasertkulsak *et al*., 2018); therefore, there was no serious membrane fouling observed during the whole operation period of the MBR in this study.

The attached biomass on the sponge cubes mainly appeared within the pores. There was almost no biofilm formation covering the outer surface of the sponges due to the physical scouring effect during their movement insider the reactor. Nevertheless, the presence of sponge media helped increase the total biomass in the system while reducing the suspended biomass concentration, which may potentially cause membrane fouling problems. In this study, the attached biomass on the sponge media (which occupied 10 % of the reactor volume) accounted for about 40 % of total biomass in the system.

Treatment performance of the MBR

The influent and effluent characteristics of the MBR operated under the presence of suspended biomass (1st condition) and integration of suspended biomass and attached biomass on sponge media (2nd condition) are shown in Table 1 and Table 2. While the influent contained organic concentrations of 139-174 $mg{\cdot}L^{-1}$ for BOD, 226-235 $mg{\cdot}L^{-1}$ for COD, and 58.4-62.5 $mg{\cdot}L^{-1}$ for TOC, there were no significant differences in their removal rates observed during the MBR operation under both conditions ($p>0.05$). Average BOD, COD, and TOC removal rates were 99.3 %, 84.6 % and 87.0 % under the 1st condition and 98.3 %, 85.2 %, and 86.0 %

under the 2nd condition, respectively. The presence of sponge media provided negligible impact on organic removal in the MBR. Meanwhile, the food-to-microorganism (F/M) ratios under both conditions were maintained in the range of 0.176-0.282 g COD $\cdot g^{-1}$ MLSS$\cdot day^{-1}$. High organic removal observed during this study could also be due to the high biodegradability of aquaculture wastewater, having a high BOD/COD ratio of 0.6-0.7. Nguyen *et al*. (2016) also reported similar COD removal efficiencies (about 84 %) in sponge-MBR and conventional MBR operated under low loading conditions.

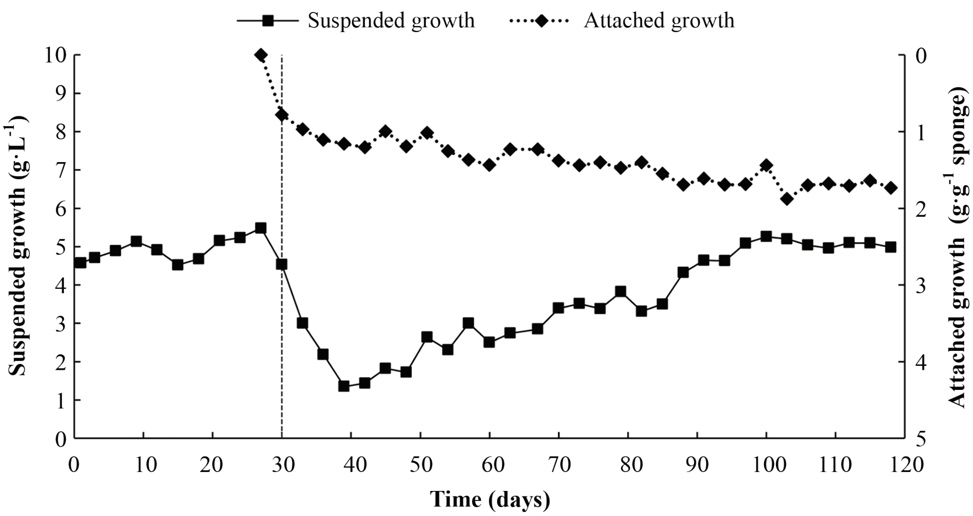

Figure 3. Suspended and attached biomass concentrations during operation of MBR.

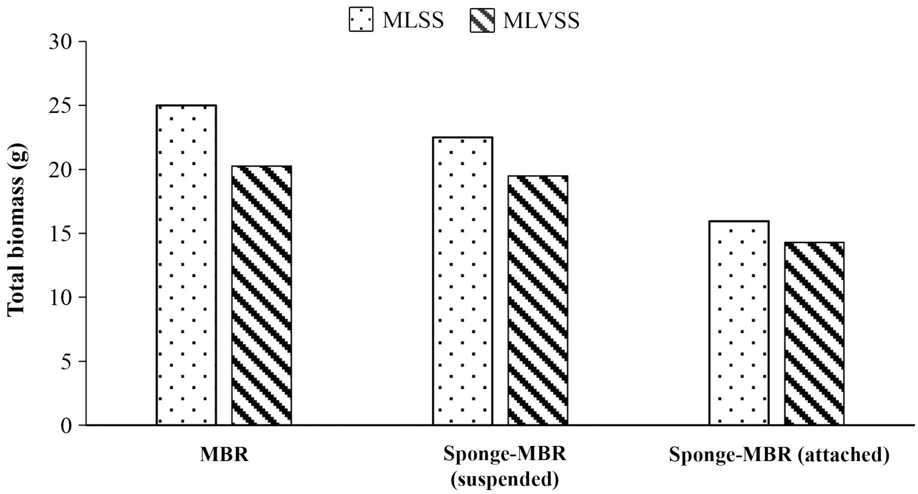

Figure 4. Total biomass in MBR during the 1st (MBR) and 2nd (sponge-MBR) conditions.

Table 2. Effluent quality and removal efficiencies of MBR operated under different conditions.

Parameters	1st condition (N = 10)		2nd condition (N = 10)	
	Effluent	R (%)	Effluent	R (%)
BOD ($mg{\cdot}L^{-1}$)	1.2±1.0	99.3	2.5±3.9	98.3
COD ($mg{\cdot}L^{-1}$)	36.6±22.2	84.6	33.5±23.9	85.2
TOC ($mg{\cdot}L^{-1}$)	8.1±2.2	87.0	7.9±5.6	86.0
SS ($mg{\cdot}L^{-1}$)	1.8±0.7	96.4	0.7±1.3	98.6
NH_4^+-N ($mg{\cdot}L^{-1}$)	0.2± 0.1	97.7	0.2±0.01	98.5
TKN ($mg{\cdot}L^{-1}$)	0.6±0.3	91.1	0.8±0.5	88.3
NO_2^--N ($mg{\cdot}L^{-1}$)	0.1±0.06	-	0.2±0.1	-
NO_3^--N ($mg{\cdot}L^{-1}$)	3.4±0.4	-	2.4±0.6	-
TN ($mg{\cdot}L^{-1}$)	3.7±1.0	38.7*	2.4±0.84	53.4*

Note: N = number of samples; values are mean±SD; R = removal percentage (%)
* denotes significant difference between conditions ($p<0.05$)

For nitrogen, the NH_4^+ and TKN removal rates under both conditions were 97.7-98.5 % and 88.3-91.1 %, respectively, and not significantly different ($p>0.05$) between conditions. Nevertheless, there was a significant difference in NO_3^- concentrations observed in the effluent ($p<0.05$). In the 1st condition, up to 73 % of NH_4^+ was oxidized, producing maximum observed NO_3^- concentration of 3.8 mg $\cdot L^{-1}$ in the effluent. From the nitrogen balance determination (Equation 1), it was found that 28.5 % of oxidized nitrogen was further assimilated to sludge (12.0 %) and denitrified (16.5 %) in the MBR under this condition (Figure 5). When the sponge media was added to the system under the 2nd condition, lower NO_3^- concentration (2.4 $mg{\cdot}L^{-1}$) was observed in the effluent. The lowered NO_3^- production under this condition could be attributed to enhanced denitrification reactions in the presence of sponge media. It led to an improvement in TN removal of about 15 % from that under the 1st condition. As a result, the observed TN removal in the MBR was increased from 38.7 % to 53.4 %. Meanwhile, NO_2^- was kept at very low concentrations of less than 0.01 mgL^{-1} under both conditions.

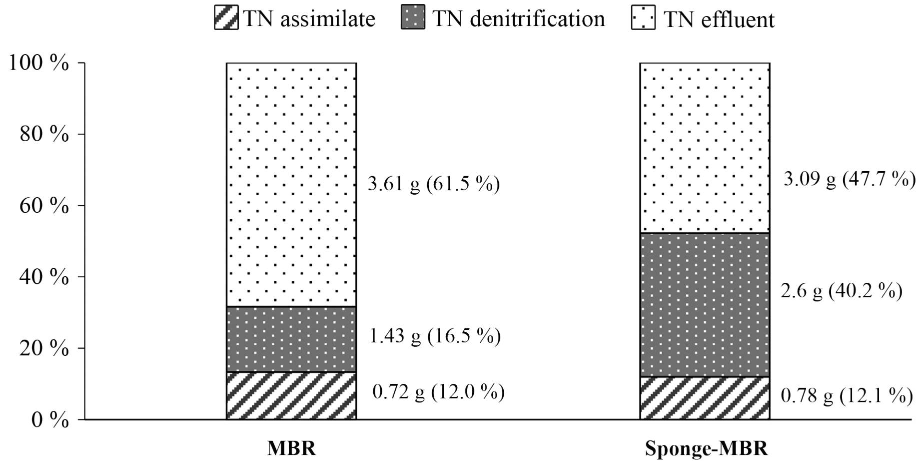

Figure 5. TN removal mechanisms in MBR and sponge-MBR.

The improved TN removal under the 2nd condition could be associated with the occurrence of simultaneous nitrification and denitrification (SND) reactions in the moving media even though the reactor was operated under aerobic conditions (Sandip and Kalyanraman, 2019). As shown in Figure 5, 40.2 % of oxidized nitrogen was denitrified under the presence of attached biomass as compared to 16.5 % under the 1st condition, thus yielding lower TN concentrations in the effluent. SND may occur in biofilm-containing reactors where the combination of nitrifying activity, anoxic niches, and COD availability may result in the growth of heterotrophic denitrifiers in the inner biofilm layer, even with bulk liquid DO maintained as high as 4 $mg \cdot L^{-1}$ (Sandip and Kalyanraman, 2019). Khan *et al*. (2011) also reported an increase in TN removal from 74 % in suspended-growth MBR to 89 % in attached-growth MBR when 15 % of PU sponge was employed in the latter reactor. When the sponge cubes are incorporated as attached growth media in the bioreactor, nitrification possibly takes place on their surface, whereas denitrification takes place in the anoxic environment created inside the sponge bodies (Nguyen *et al*., 2010).

Nevertheless, there were some differences in the degree of membrane fouling observed in the MBR operated in the absence and presence of sponge media. The development of transmembrane pressure was observed on some days during the MBR operation as the SS were removed from the effluent by membrane filtration. The incoming suspended solids consisted of fine particles originally present in the fishpond water and the digested fish feed with particle size smaller than 5 µm. They were almost completely retained by the submerged membrane with removal efficiencies of 96.4-98.6 %. Moreover, biological solids present in the mixed liquor were also completely retained in the reactor through membrane filtration.

Nitrification and denitrification activity of biomass

The nitrification and denitrification capacities of suspended, attached, and combined biomass examined in batch experiments are presented in Table 3. In terms of nitrification, the NH_4^+ removal by suspended and attached biomass was 26.0 % and 13.1 %, respectively, during 4 h reaction time, while removal improved to 37.1 %

Table 3. Nitrification and denitrification capacities of biomass in batch experiments.

Parameters	Suspended biomass	Attached biomass	Combined biomass
Nitrification			
Initial NH_4^+-N ($mg \cdot L^{-1}$)	47.3	47.3	47.3
Final NH_4^+-N ($mg \cdot L^{-1}$)	35.0	41.1	29.8
Final NO_3^--N ($mg \cdot L^{-1}$)	7.5	4.9	8.9
NH_4^+ removal (%)	26.0	13.1	37.1
Volumetric nitrification rate ($mg \cdot L^{-1} \cdot h^{-1}$)	3.4	1.9	4.6
Biomass weight used in batch experiment (g)	0.37	0.28	0.65
Specific nitrification rate of biomass ($mg \cdot g^{-1} \cdot h^{-1}$)	2.1	1.4	1.7
First-order nitrification rate (h^{-1})	0.21	0.12	0.23
Denitrification			
Initial NO_3^- ($mg \cdot L^{-1}$)	17.3	17.3	17.3
Final NO_3^- ($mg \cdot L^{-1}$)	15.9	14.7	13.5
NO_3^- removal efficiency (%)	9.2	13.7	20.2
COD removal efficiency (%)	54.4	60.9	73.9
Volumetric denitrification rate ($mg \cdot L^{-1} \cdot h^{-1}$)	0.4	0.7	1.4
Biomass weight used in batch experiment (g)	0.37	0.28	0.65
Specific denitrification rate of biomass ($mg \cdot g^{-1} \cdot h^{-1}$)	0.23	0.58	0.36
First-order denitrification rate (h^{-1})	0.06	0.14	0.18

when a combination of suspended and attached biomass was applied. Correspondingly, volumetric nitrification rates were determined as 3.4, 1.9 and 4.6 $mg \cdot L^{-1} \cdot h^{-1}$, respectively. Meanwhile, biomass-specific nitrification rates were determined as 2.1, 1.4 and 1.7 $mg \cdot g^{-1} \cdot h^{-1}$, respectively. Obviously, the presence of biomass in both forms when the MBR was operated with sponge media helped increase the volumetric nitrification rate despite having a lower specific nitrification rate. Of the two forms, the suspended biomass had higher nitrification rate than the attached biomass, as the former was exposed to higher oxygen availability. Even though there was a significant difference in nitrification capacities between the suspended and attached biomass in the MBR, there was no difference in observed nitrification degree between the 1^{st} and 2^{nd} conditions, as the MBR was operated at much lower nitrogen loading than in batch tests.

In the denitrification test, NO_3^- removal rates were 9.2 % and 13.7 %, corresponding to volumetric denitrification rates of 0.4 and 0.7 $mg \cdot L^{-1} \cdot h^{-1}$ for suspended and attached biomass, respectively. Meanwhile, the use of integrated biomass promoted NO_3^- removal to 20.2 % and denitrification rate to 1.4 $mg \cdot L^{-1} \cdot h^{-1}$. The specific denitrification rates of suspended, attached and integrated biomass were determined as 0.23, 0.58 and 0.36 $mg \cdot g^{-1} \cdot h^{-1}$. Not surprisingly, the denitrification rates observed under aerobic conditions were found to be much lower than nitrification rates, as former the reaction depends on the presence of anoxic conditions in the biomass. Meanwhile, attached biomass had higher denitrification rate compared to suspended biomass, as a DO gradient existed from the surface towards the inner part of the sponge, where the lower oxygen concentration was more suitable for denitrification (He *et al*., 2009). Chae *et al*. (2012) studied the spatial distribution of nitrogen-transforming bacteria in sponge media of a biological nutrient removal plant and revealed the presence of nitrifying bacteria on the surface of the media, whereas denitrifying and anaerobic ammonium oxidizing bacteria were detected within the inner part of sponge cubes; furthermore, their coexistence played an important role in nitrogen removal. Denitrification capacity of biomass is also associated with the availability of carbon sources present in the wastewater (Peng *et al*., 2007). Theoretically, it was determined that the amount of organic substrate (184 $mg\ COD \cdot L^{-1}$) was sufficiently available for denitrification in the batch experiments (Sobieszuk and Szewczyk, 2006); thus, denitrification capacities would depend mainly on nitrate concentration and the availability of anoxic conditions within the biomass. The addition of sponge media into the MBR could promote TN removal efficiency through increased denitrification sites while maintaining observed nitrification efficiency at the same level as the suspended biomass reactor.

The first-order rate constants of nitrification and denitrification reactions of the suspended, attached and combined biomass are also presented in Table 3. For nitrification, the highest rate constant was observed in the case of combined biomass (0.23 h^{-1}), followed by the suspended (0.21 h^{-1}) and attached biomass (0.12 h^{-1}). Meanwhile, the denitrification rate constants of suspended, attached and combined biomass were 0.063, 0.14 and 0.18 h^{-1}, respectively.

According to the aforementioned results, simultaneous nitrification and denitrification could proceed in the MBR operated under aerobic conditions, thus biological nitrogen removal could be achieved. When the sponge media was incorporated into the MBR, higher nitrogen removal was observed through the increase in biomass as well as the presence of an anoxic zone inside the attached biomass. Meanwhile, there was no adverse impact on the nitrification level in the MBR operated under low loading conditions in this study. Considering the satisfactory performance of MBR in terms of organic and nitrogen removal achieved in this study, it is proposed that the MBR with sponge media can be considered as an alternative treatment system for controlling water pollution in a recirculating aquaculture system.

CONCLUSION

This research compared the performance of an MBR operated with and without sponge media for the treatment of aquaculture wastewater. The results demonstrate that total nitrogen removal improved significantly from 38.7 % to 53.4 % when sponge media was introduced in the MBR without affecting organic removal or nitrification efficiency. High BOD and NH_4^+ removal rates of 98.3 % and 98.5 % were achieved. The incorporation of sponge media, however, improved TN removal through the presence of anoxic conditions in the attached biomass which promoted simultaneous nitrification-denitrification reactions despite the MBR being operated with continuous aeration. During steady operation, the biomass distribution was 58.5 % in suspended form and 41.5 % in attached form. Batch experiments confirmed higher nitrification rate by suspended biomass (2.1 $mg \cdot g^{-1} \cdot h^{-1}$) and higher denitrification rate by attached biomass on sponge media (0.58 $mg \cdot g^{-1} \cdot h^{-1}$). Thus, the use of both suspended and attached biomass together promoted favorable nitrification and denitrification capacity and reaction rates in the MBR.

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LITERATURE CITED

American Public Health Association (APHA). 2012. **Standard Methods for the Examination of Water and Wastewater,** 22nd ed. American Public Health Association, Washington, D.C., USA. 724 pp.

Ammary, B.Y. 2004. Nutrients requirements in biological industrial wastewater treatment. **African Journal of Biotechnology** 3(4): 236–238.

Bôto, M., C.M.R. Almeida and A.P Mucha. 2016. Potential of constructed wetlands for removal of antibiotics from saline aquaculture effluents. **Water** 8: 465. DOI: 10.3390/w8100465.

Cao, L., W. Wang, Y. Yang, C. Yang, Z. Yuan, S. Xiong and J. Diana. 2007. Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. **Environmental Science and Pollution Research** 14: 452–462.

Chae, K.J., S.M. Kim, S.E. Oh, X. Ren, J. Lee and I.S. Kim. 2012. Spatial distribution and viability of nitrifying, denitrifying and ANAMMOX bacteria in biofilms of sponge media retrieved from a full-scale biological nutrient removal plant. **Bioprocess and Biosystems Engineering** 35: 1157–1165.

Chiam, C.K. and R. Sarbatly. 2011. Purification of aquacultural water: conventional and new membrane-based techniques. **Separation and Purification Reviews** 40: 126–160.

Chiemchaisri, C. and C. Liamsangoun. 2004. Simultaneous organic stabilization and nitrogen removal in multi-stage biodrum system. **Water Science and Technology** 50(6): 95–101.

Chu, L. and J. Wang. 2011. Comparison of polyurethane foam and biodegradable polymer as carriers in moving bed biofilm reactor for treating wastewater with a low C/N ratio. **Chemosphere** 83: 63–68.

Crab, R., Y. Avnimelech, T. Defoirdt, P. Bossier and W. Vestraete. 2007. Nitrogen removal techniques in aquaculture for a sustainable production. **Aquaculture** 270: 1–14.

Dong, Z., M. Lu, W. Huang and X. Xu. 2011. Treatment of oilfield wastewater in moving bed biofilm reactors using a novel suspended ceramic biocarrier. **Journal of Hazardous Materials** 196: 123–130.

Eding, E.H., A. Kamstra, J.A.J. Verreth, E.A. Huisman and A. Klapwijk. 2006. Design and operation of nitrifying trickling filters in recirculating aquaculture. **Aquacultural Engineering** 34: 234–260.

Gentili, A.R., M.A. Cubitto, M. Ferrero and M.S. Rodriguéz. 2006. Bioremediation of crude oil polluted seawater by a hydrocarbon degrading bacterial strain immobilized on chitin and chitosan flakes. **International Biodeterioration and Biodegradation** 57: 222–228.

Guo, W., H.H. Ngo, F. Dharmawan and C.G. Palmer. 2010. Roles of polyurethane foam in aerobic moving and fixed bed bioreactors. **Bioresource Technology** 101: 1435–1439.

He, S.B., G. Xue and B.Z. Wang. 2009. Factors affecting simultaneous nitrification and de-nitrification (SND) and its kinetics model in membrane bioreactor. **Journal of Hazardous Materials** 168: 704–710.

Iorhemen, O.T., R.A. Hamza and J.H. Tay. 2016. Membrane bioreactor (MBR) technology for wastewater treatment and reclamation: membrane fouling. **Membranes** 6: 33. DOI: 10.3390/membranes6020033.

Jin, Y., D. Ding, C. Feng, S. Tong, T. Suemura and F. Zhang. 2012. Performance of sequencing batch biofilm reactors with different control systems in treating synthetic municipal wastewater. **Bioresource Technology** 104: 12–18.

Khan, S.J., S. Ilyas, S. Javid, C. Visvanathan and V. Jegatheesan. 2011. Performance of suspended and attached growth MBR systems in treating high strength synthetic wastewater. **Bioresource Technology** 102: 5331–5336.

Kõiv, M., K. Mahadeo, S. Brient, D. Claveau-Mallet and Y. Comeau. 2016. Treatment of fish farm sludge supernatant by aerated filter beds and steel slag filters-effect of organic loading rate. **Ecological Engineering** 94: 190–199.

Konnerup, D., N.T.D. Trang and H. Brix. 2011. Treatment of fishpond water by recirculating horizontal and vertical flow constructed wetlands in the tropics. **Aquaculture** 313: 57–64.

Lim, J.W., P.E. Lim and C.E. Seng. 2012. Enhancement of nitrogen removal in moving bed sequencing batch reactor with intermittent aeration during REACT period. **Chemical Engineering Journal** 197: 199–203.

Lindholm-Lehto, P., J. Pulkkinen, T. Kiuru, J. Koskela and J. Vielma. 2020. Water quality in recirculating aquaculture system using woodchip denitrification and slow sand filtration. **Environmental Science and Pollution Research** 27: 17314–17328.

Liu, Q., X.C. Wang, Y. Liu, H. Yuan and Y. Du. 2010. Performance of a hybrid membrane bioreactor in municipal wastewater treatment. **Desalination** 258: 143–147.

Ngo, H.H., W. Guo and X. Xing. 2008. Evaluation of a novel sponge submerged membrane bioreactor (SSMBR) for sustainable water reclamation. **Bioresource Technology** 99: 2429–2435.

Nguyen, T.T, H.H. Ngo, W. Guo, A. Johnston and A. Listowski. 2010. Effects of sponge size and type on the performance of an up-flow sponge bioreactor in primary treated sewage effluent treatment. **Bioresource Technology** 101: 1416–1420.

Nguyen, T.T., X.T. Bui, D.D. Nguyen, P.D. Nguyen, H.H. Ngo and W. Guo. 2016. Performance and membrane fouling of two types of laboratory-scale submerged membrane bioreactors for hospital wastewater treatment at low flux condition. **Separation and Purification Technology** 165: 123–129.

Peng, Y.Z., M. Yong and S.Y. Wang. 2007. Denitrification potential enhancement by addition of external carbon sources in a pre-denitrification process. **Journal of Environmental Sciences** 19: 284–289.

Prasertkulsak, S., C. Chiemchaisri and W. Chiemchaisri. 2018. Pharmaceutical compound removal during mixed liquor filtration in membrane bioreactor operated under long sludge age. **Jurnal Teknologi** 80: 45–50.

Sandip, M. and V. Kalyanraman. 2019. Enhanced simultaneous nitri-denitrification in aerobic moving bed biofilm reactor containing polyurethane foam-based carrier media. **Water Science and Technology** 79(3): 510–517.

Sobieszuk, P. and K.W. Szewczyk. 2006. Estimation of (C/N) ratio for microbial denitrification. **Environmental Technology** 27: 103–108.

Thanh, B.X., H. Berg, L.N.T. Nguyen and C.T. Da. 2013. Effects of hydraulic retention time on organic and nitrogen removal in a sponge-membrane bioreactor. **Environmental Engineering Science** 30(4): 194–199.

Yang, S., F. Yang, Z. Fu and R. Lei. 2009. Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on organic carbon and nitrogen removal. **Bioresource Technology** 100: 2369–2374.

Yang, S., F. Yang, Z. Fu, T. Wang and R. Lei. 2010. Simultaneous nitrogen and phosphorus removal by a novel sequencing batch moving bed membrane bioreactor for wastewater treatment. **Journal of Hazardous Materials** 175: 551–557.

Yin, J., P. Zhang, F. Li, G. Li and B. Hai. 2015. Simultaneous biological nitrogen and phosphorus removal with a sequencing batch reactor biofilm system. **International Biodeterioration and Biodegradation** 103: 221–226.

Zou, S., L. Guan, D.P. Taylor, D. Kuhn and Z. He. 2018. Nitrogen removal from water of recirculating aquaculture system by a microbial fuel cell. **Aquaculture** 497: 74–81.