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Treatment of aquaculture farm effluent containing antibiotics in high-rate membrane bioreactor

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ABSTRACT

High-rate membrane bioreactor (MBR) was applied for simultaneous removal of organic substances, nitrogen and antibiotics, i.e., amoxicillin (AMX), sulfadiazine (SDZ) and trimethoprim (TMP) in aquaculture farm effluent. The operation of MBR was carried out under different conditions, i.e., (1) no sludge wastage, (2) periodical sludge wastage to control sludge concentration at 5 g L^{-1} and (3) addition of sponge media (10% v/v) as hydraulic retention time was kept constant at 4 h. While high biochemical oxygen demand, total Kjeldahl nitrogen and NH_3 removal of 85% or more were consistently achieved under all operating conditions, there was a large variation in antibiotic removal among the studied antibiotics ranging from >99% for AMX to 36% for SDZ. Biomass control in the MBR through sludge withdrawal did not have adverse impact on AMX and TMP removal as they were mainly removed through biodegradation but reduced the removal of recalcitrant compound, i.e., SDZ, by 10%. Moreover, membrane filtration could also help retaining residual antibiotics within the MBR.

Keywords: Antibiotics; Aquaculture effluent; MBR; HRT

1. Introduction

Aquaculture has become the fast-growing industry all over the world due to the increasing food demand. For instance, total fish supply has been projected to increase from 154 million tons in 2011 to 186 million tons in 2030 [1]. The operation of aquaculture farms varies from completely open (cages, pens) to almost completely closed recirculating systems, contributing different pollutant loadings to natural environment [2]. In general practice, natural pond is commonly used for the cultivation of fish, shrimp and shellfish especially for inland aquaculture production in Asia [3]. To prevent any infectious disease and increase the growth of aquatic organisms, the therapeutic and non-therapeutic antibiotics, i.e., β-lactam, sulfonamides and quinolone, are widely used [4]. Those antibiotics are commonly mixed with the feed and then periodically applied to the pond during the process of cultivation [5]. This feeding process also leads to the contamination of pond water with suspend solids, nitrogenous compounds, residual antibiotics, and some dissolved organic matter due to the accumulation of feed residue and fish excreta. Those pollutants further result in detrimental effect to the surrounding ecosystem when the polluted water is directly discharged to the environment without proper treatment [6]. It was reported that nutrient quantities discharged together with the effluent from aquaculture farms may differ by a factor up to 10 depending on aquaculture species or production systems [7]. These differences in emission would yield different eutrophic degree in natural water and toxicity level to aquatic organisms. Furthermore, residual antibiotics at even low concentration in the river could provide adverse impact to microbe or aquatic organisms [8]. For instance, sulfonamides have been reported to create obvious toxicity condition to various organisms, i.e., *Daphnia magna*, *Sparus aurata* L. and

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Caenorhabditis elegans [9–11]. The residual antibiotic could also exert selective pressure to the bacteria population to acquire antibiotic resistance [12]. Moreover, those effects will become more harmful to human because surface water such as river and drainage water is widely used as water source for drinking and irrigation purposes [13].

The solutions to nutrient and antibiotic pollutions from aquaculture were proposed. Biological treatment including rotating biological contactor, trickling filter, bead filters and fluidized sand biofilters are conventionally used in intensive aquaculture systems [14]. The biological wastewater treatment technologies are considered as primary barrier of the antibiotics because they played an important role in preventing the residual antibiotics to the surface water. However, most conventional biological treatment systems are not efficient in removing antibiotics from wastewater effluents and constructed wetland with interactions among soil/sediment, plant and microorganisms were considered as potential solution [15], but it would still require large treatment area [16]. During the past decades, the advanced biological wastewater treatment coupled with membrane filtration, commonly called, membrane bioreactor (MBR) has become a promising state-of-the-art technology for the treatment of wastewater containing organic, nitrogen and antibiotics [17]. However, membrane fouling is still an obstacle to overcome especially when the MBR was operated under short hydraulic retention time (HRT) condition and high membrane permeate flux rate. The use of sponge media as moving carrier in MBR could promote rapid and stable growth of attached biomass [18] thus reduce membrane fouling from suspended biomass. Moreover, incorporation of attached biomass on media into single-stage aerobic MBR could also improve nitrogen removal through promotion of simultaneous nitrification and denitrification reactions [19]. For antibiotics removal, previous research [20] revealed that the influent antibiotics entering the MBR system were significantly removed through biodegradation while immediate adsorption onto colloidal particles supernatant of MBR sludge and subsequent rejection by membrane filtration also played important role for the removal of recalcitrant compounds. Compared to conventional treatment system, the MBR system operated at high solid retention time (SRT) could highly remove residual antibiotics from wastewater due to its higher sludge concentration with enrichment of slowly growing specific microorganisms such as nitrifiers [21]. Higher antibiotic removal efficiencies were mostly reported in the MBR operated under high biomass concentrations [22]. Nevertheless, it is essential to control biomass sludge concentration properly in order to prevent membrane fouling especially when high-rate MBR was operated under short HRT or high organic loading rate. However, the effect of biomass control through sludge wastage operation on antibiotic removal is inconclusive. Moreover, the biodegradation kinetics of antibiotics in high-rate MBR operated under low and high sludge concentrations have not been previously reported. Recent research [23] also reported that usual kinetics which consider that the removal rates of pharmaceutical compounds are proportional to biomass concentration are only valid for low and moderate biomass concentrations and kinetic model should be developed for wide spectrum of biomass concentrations. Therefore, this research work aims at applying MBR under different biomass conditions for the treatment of aquaculture farm effluent containing antibiotics. The antibiotic removal kinetics of MBR sludge operated under no sludge wastage (high biomass) and sludge wastage (low biomass) conditions were investigated.

2. Materials and methods

2.1. Laboratory scale membrane bioreactor and operating conditions

A laboratory scale MBR consisting of 5 L volume was used in this study (Fig. 1). The tank is made of acrylic with dimension of 15 × 10 × 45 cm. Three modules of submerged hollow-fiber membrane (Sterapore SADF™, Polyvinylidene fluoride (PVDF), 0.4 μm pore size, 0.105 m^2 of surface area) were immersed into the reactor. Aeration supplied in MBR tank was 9 L min^{-1} yielding constant dissolved oxygen (DO) of 5–6 mg L^{-1}. HRT in MBR was kept constantly at 4 h, yielding a constant permeate flow rate of 30 L d^{-1}. To define the appropriate operating condition, the experiments were carried out under three different conditions, i.e., 1st condition: no sludge wastage (week 1–34), 2nd condition: under constant sludge concentration of 5 g L^{-1} (week 35–51) through regular sludge withdrawal and 3rd condition: when polyurethane (PU) sponge cube media at 10% of reactor volume was placed (week 52–81). The PU sponge density was 22 kg m^{-3} and it was prepared in cubic shape of 1 × 1 × 1 cm size.

2.2. Feeding wastewater and analysis of samples

The aquaculture farm effluent was prepared by mixing 60 g of fish feed with 100 L of pond water to simulate actual aquaculture farm effluent in Thailand. The major antibiotics found in aquaculture farm effluent were amoxicillin (AMX), trimethoprim (TMP) coupled with sulfadiazine (SDZ) and their concentrations were set at 100 μg L^{-1} representing their upper limit of observed concentrations.

The physicochemical characteristics of feeding wastewater are shown in Table 1. The analyses of influent and

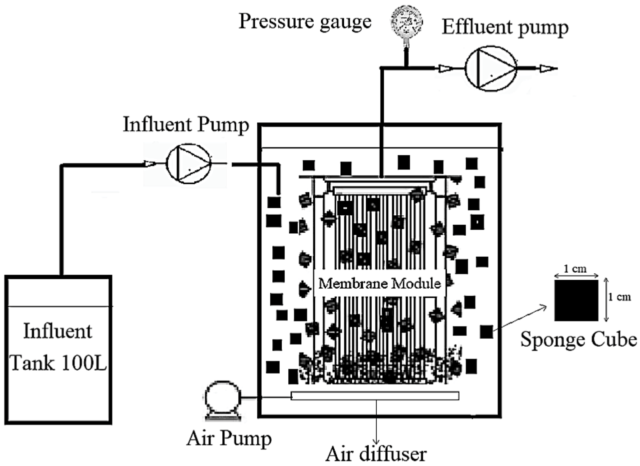

Fig. 1. Schematic of MBR system incorporated with sponge media.

effluent samples were performed twice a week. The water quality parameters including suspended solids (SSs), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen (NH_3–N), nitrite nitrogen (NO_2–N), nitrate nitrogen (NO_3–N) and total Kjeldahl nitrogen (TKN) were analyzed in triplicate following Standard Methods for the Examination of Water and Wastewater [24]. Total nitrogen (TN) was calculated from summation of NH_3–N, NO_2–N, NO_3–N and organic nitrogen whereas organic nitrogen was determined from the difference between TKN and NH_3–N. In addition, pH was measured by pH meter (SI Analytics Lab 855) and total organic carbon (TOC) was analyzed by TOC analyzer (Shimadzu). During the reactor operation, sludge concentrations were determined in terms of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). In the 3rd condition, the attached sludge on the PU sponge was also determined by rinsing the solids from sponge media with deionized water following the procedures described in previous research [25].

AMX, TMP and SDZ concentrations in the influent, effluent and sludge were analyzed in triplicate following standard extraction procedures [26] using Oasis HLB cartridge (Waters, USA) and antibiotic concentrations were analyzed by high-performance liquid chromatography equipped with an ultraviolet (UV) detector (Shimadzu, Japan). For all targeted antibiotics analyses, the mobile phase consists of a 95% phosphate buffer (0.01 mol L^{-1}), pH = 5.5 and 5% of acetonitrile mixture. The mobile phase flow rate of 1.0 ml min^{-1} and UV detection at 200 nm were used. The temperature of the detector used was 40°C. The lower limit detection of antibiotics was 1 μg L^{-1} and the coefficient of determination of calibration curve of all targeted standard antibiotics was higher than 0.98. The standard antibiotics with the purity of 99% or higher were purchased from Sigma-Aldrich (USA).

2.3. Study of antibiotics removal mechanisms in batch experiments

To clarify antibiotic removal mechanisms in MBR, batch experiments were performed to determine the removal of antibiotics through adsorption and biodegradation by MBR sludge obtained during the steady reactor operation. The batch experiment was performed in a serum bottle with a sample volume of 100 mL. The serum bottle was covered by aluminum foil to prevent possible photo-degradation. The aerobic reactor sludge with concentration of 2 g L^{-1} was investigated in batch experiments under aerobic conditions. The initial AMX, TMP and CTC concentrations used in all batch experiments were set at 1,000 μg L^{-1}. All the experiments were carried out in duplicate with triplicate analyses of each sample while a control experiment conducted using synthetic wastewater and antibiotic compounds without the presence of sludge were also performed. To distinguish the removal through adsorption and biodegradation, parallel experiments under the same conditions as mentioned above were conducted using aerobic sludge samples autoclaved under 121°C for 15 min thrice to quantify the amount of antibiotics removed through adsorption on the sludge particles. Each batch experiment was performed over 12 h during which representative samples were collected at every 2 h interval. The differences in antibiotic removal using active and inactive sludge were considered as the removal through biodegradation. Moreover, the biodegradation rate constants of studied antibiotics by aerobic sludge were derived by following the first-order kinetic expression [27].

2.4. Statistical analysis

Microsoft Excel version 2010 was used for the statistical analysis in this experimental data. The significant difference (p < 0.05 in one-way ANOVA analysis) between the water quality parameters and antibiotic concentrations under each condition was analyzed.

3. Results and discussion

3.1. Treatment performance of the membrane bioreactor

The treatment performance of MBR under all operating conditions is given in Table 1. There was no significant difference (p > 0.05) in pH among all operating conditions.

Table 1
Characteristic of polluted water and removal performance of MBR

Parameter	1st condition (week 1–34, n = 34)			2nd condition (week 35–52, n = 17)			3rd condition (week 52–81, n = 29)		
	Influent	Effluent	Removal %	Influent	Effluent	Removal %	Influent	Effluent	Removal %
pH	6.7 (0.3)	7.8 (0.5)	—	6.6 (0.2)	7.6 (0.2)	—	6.4 (0.3)	7.3 (0.2)	—
SS (mg L^{-1})	61.6 (46.2)	1.5 (0.7)	95.3	49.5 (23.7)	1.8 (0.7)	97.0	51.2 (16.9)	0.7 (1.3)	98.6
BOD (mg L^{-1})	115.6 (50.8)	2.1 (1.8)	98.6	173.6 (29.2)	1.2 (7.2)	99.3	138.7 (28.3)	2.5 (3.9)	98.3
COD (mg L^{-1})	181.7 (77)	34.9 (21.3)	85.0	235.0 (37.9)	34.6 (22.2)	84.6	226.4 (55.1)	33.5 (23.9)	85.2
TOC (mg L^{-1})	62.9 (17.8)	7.2 (2.4)	80.3	62.5 (3.9)	8.1 (2.2)	87.0	58.4 (6.7)	7.9 (5.6)	86.0
NH_3–N (mg L^{-1})	4.9 (3.1)	0.4 (0.8)	93.6	4.1 (2.5)	0.4 (0.2)	90.0	5.2 (0.1)	0.2 (0.01)	98.5
TKN (mg L^{-1})	9.4 (5.1)	1.1 (1.0)	87.3	9.7 (5.2)	3.6 (1.3)	86.1	7.1 (1.1)	0.8 (0.5)	88.3
NO_2–N (mg L^{-1})	0.2 (0.1)	0.7 (1.3)	—	0.3 (0.1)	0.3 (0.2)	—	0.1 (0.01)	0.2 (0.1)	—
NO_3–N (mg L^{-1})	0.1 (0.02)	4.5 (1.9)	—	0.5 (0.2)	4.2 (0.5)	—	0.1 (0.05)	2.4 (0.6)	—
TN (mg L^{-1})	9.7 (5.2)	6.3 (5.2)	35.0	10.5 (5.5)	8.1 (2.0)	22.8	7.2 (1.1)	3.4 (0.8)	53.4

pH was constantly kept under neutral condition (6.4–7.8) while all suspended particles incoming with feeding wastewater highly removed by submerged membrane modules (>95%). In the 1st condition, MLSS increased from 0.2 g L^{-1} at week 1 to 8.5 g L^{-1} at week 34. During this period, the sludge production yield was determined at 0.25 g MLSS g^{-1} BOD removed. Meanwhile, the MLVSS and MLSS ratios were kept between 0.78 and 0.85. This microbial sludge increase took place during the microbial growth in the system operated without sludge wastage. The enrichment of biomass in MBR led to an improvement of organic and nitrogen removal along the operation period of 34 weeks. During this period, average COD, BOD and TOC removal was 80.3%, 96.6% and 85.0% while NH_3–N, TKN and TN of 93.6%, 87.3% and 35.0% was removed, respectively. Up to 90% of NH_3–N was oxidized to NO_3–N, yielding its product of 4.5 mg L^{-1} in the effluent. Only approximately 10% of oxidized was further denitrified under aerobic condition but the denitrification was gradually improved as the sludge concentration in the MBR increased and reached its maximum level of 8.5 g L^{-1}. This improvement could be explained by the formation of anoxic zone for denitrification inside sludge particle where oxygen penetration would be limited especially at higher sludge concentration [28].

Despite high BOD and TKN removal achieved in the 1st condition, an increase in biomass solids started to signal adverse impact on membrane filtration noticeable in terms of transmembrane pressure development. The membrane fouling was mainly caused by the formation of sludge cake on the membrane surface leading to an increase in transmembrane pressure from 5 to 16 kPa d^{-1}. Thus, membrane cleaning through physical removal of attached solids from the membrane surface was regularly performed on weekly basis to maintain the constant permeate flux at 30 L d^{-1}. In the 2nd condition, the operation of MBR under constant sludge concentration of 5 g L^{-1} was examined. To control sludge concentration, approximately 200 mL of sludge was daily drained out from the reactor. Under such operating condition, average SRT in MBR was estimated at 25 d. In terms of treatment performance, there were no significant difference in organic removal in terms of BOD, COD and TOC ($p > 0.05$) between the 1st and the 2nd conditions as the organic removal were maintained at 85% or above. Meanwhile, nitrogen removal in terms of NH_3–N and TN were slightly decreased to 90% and 22.8% while TKN removal was remained relatively constant (86%). The reduction of NH_3–N and TN removal was possibly caused by the decrease of nitrification activities through sludge wastage. Moreover, the operation at low sludge concentration of 5 g L^{-1} could also reduce anoxic zone in the sludge particles thus limited denitrification site. Nevertheless, the MBR operation could still produce the effluent with indifferent level of oxidized nitrogen from that of the 1st condition (4.2 mg L^{-1}).

In the 3rd condition, PU sponge media was incorporated into the MBR. It was found that sludge concentration in MBR was gradually decreasing from 5.4 g L^{-1} at week 52 to 1.3 g L^{-1} at week 55. Afterward, sludge concentration gradually increased to 4 g L^{-1} at week 81. The changes in suspended sludge concentration were influenced by initial attachment of solids to PU sponge media followed by its detachment when saturation of biomass attachment on the media has been reached. At steady condition, attached sludge on the sponge media were determined at 1.3 g g^{-1} sponge and the attached sludge accounted for 47% of total sludge (combined suspended and attached sludge) in the system. Even though higher sludge concentration in the MBR was observed under this operating condition, the introduction of PU sponge helped maintaining transmembrane pressure less than 5 kPa throughout the whole operation period. Considering the treatment performance, there was no significant difference in organic removal (BOD, COD) between the 3rd condition and previous operating conditions ($p > 0.05$). In contrast, NH_3–N and TKN removal were slightly improved to 98.5% and 88.3% ($p > 0.05$) whereas TN was significantly increased to 53.4% ($p < 0.05$). From the improved TN removal, average oxidized nitrogen in the effluent was kept as low as 2.4 mg L^{-1}.

In overall, there was no significant impact on the operating conditions of MBR on the organic and nitrogen removal except TN. High treatment performance was possibly associated with high biodegradability of simulated aquaculture farm effluent as expressed in terms of high BOD/COD ratio (>0.5). The maintenance of biological sludge in the system even under sludge wastage condition allowed the development of high nitrification activities in the system. The only difference between the operating conditions was observed in terms of TN removal. The NH_3–N and TN removal were 93.6% and 35% under the 1st condition while those under the 2nd condition were only 90% and 22.8%, respectively. When PU sponge was introduced in MBR, NH_3–N removal slightly increased to 98.5% while TN increased to 53.4%. The determination of nitrification and denitrification rate in sludge under aerobic condition (DO = 5–6 mg L^{-1}) revealed that they were 3.4 and 0.4 mg L^{-1} h^{-1}, respectively, for suspended biomass whereas they were improved to 4.6 and 1.4 mg L^{-1} h^{-1} when both suspended and attached sludge was presented. As described earlier, simultaneous nitrification and denitrification reactions could take place in suspended sludge but maintenance of nitrifying microorganisms in mixed sludge and the presence of anoxic zone could be reduced under sludge wastage condition which may affect nitrifying and denitrifying capacities within the MBR. Under the presence of PU sponge, the MBR could be operated under high sludge amount without adverse impact on the membrane filtration. Higher sludge concentration and longer SRT could enhance nitrifying and denitrifying bacteria in the reactor. Nitrifying microorganisms could be developed not only in the suspended sludge, but also on the surface of the sponge biofilm [25]. The presence of DO gradient occurred along the sponge-inward depth resulting in higher anoxic reaction inside the portion of sponge media [29]. Therefore, the high-rate MBR developed in this study provided high BOD, COD and TKN removal from aquaculture farm effluent when it was operated with only suspended sludge but integration of sponge media would be required if TN level in the effluent needed to be stringently controlled.

3.2. Antibiotic removal in the membrane bioreactor

The average concentrations of studied antibiotics and their removal in the MBR under different operating conditions are presented in Table 2. As described earlier, the microbial sludge responsible for the pollutant removal was maintained in suspended form during the 1st and 2nd condition whereas there were both suspended and attached biomass in the 3rd condition. In this condition, both sludges might contribute similar to the treatment as the amount of sludge, e.g., 47% and 53% in attached and suspended forms, respectively, and their microbial characteristics are found indifferent. Therefore, adsorption phenomena of antibiotics onto microbial sludge in both forms would be similar. Thus, the removal capacities of antibiotics were investigated under different sludge concentrations of the 1st and 2nd conditions were explored. As shown in Table 2, the removal of AMX, SDZ and TMP was 100%, 42.6% and 95.6% in the 1st condition. After the treatment, the effluent contained less than 0.1 μg AMX L^{-1} while the average SDZ and TMP concentrations were 42.6 and 3.5 μg L^{-1}, respectively. There was no significant difference in AMX removal under both conditions ($p > 0.05$). In contrast, SDZ and TMP were slightly decreased by 6.2% and 1.1% but the differences were at insignificant level ($p > 0.05$). From this finding, the MBR operation at lower sludge concentration of 5 g L^{-1} did not provide adverse impact in antibiotic removal. The removal of antibiotics by MBR sludge was responsible by biodegradation, adsorption and hydrolysis [30]. Likewise, those removal processes could be influenced by organic loading, MLSS, HRT, SRT, pH and temperature could also influence the antibiotic removal [31]. During the experiment, there was no observed inhibitory effect of antibiotics to MBR sludge. Previous study [32] also suggested that there was no inhibition effect of AMX on mixed aerobic microbial consortia due to its chemical structure, polarity and long SRT operation thus creating rapid biodegradation reaction. On the contrary, toxicological effect of SDZ and TMP to nitrifying bacteria in activated sludge was reported, resulting in nitrogen transformation inhibition [33]. In MBR, their inhibition effect could be minimized due to low residual levels of antibiotics [34].

The removal of antibiotics in the MBR could be defined by their partition coefficient ($\log K_{ow}$) and water solubility. The antibiotics with low $\log K_{ow}$ and high-water solubility are defined as hydrophilic compounds while those with high $\log K_{ow}$ and low water solubility are defined as hydrophobic compounds [35]. As shown in Fig. 2, supernatant to influent (S/I) ratio of AMX was less than 0.1 under both conditions. Therefore, AMX ($\log K_{ow}$ = 0.87) was mainly removed in the MBR through biodegradation. High removal of AMX is possibly due to its structural similarity to other substances, which render to microorganisms. AMX was antibiotics classified in β-lactam class, which is rapidly degraded by hydrolytic cleavage and ultimately mineralized to carbon dioxide and water. There was no residual concentration of AMX detected in mixed liquor sludge. The effluent to supernatant (E/S) ratio of less than 0.1 also demonstrated that membrane filtration helps in retaining remaining AMX in supernatant, yielding total AMX removal up to 99%.

For SDZ, this antibiotic was found persistent to adsorption and biotransformation, showing high S/I ratio of 0.64–0.66 (Fig. 2) and its removal efficiencies were much lower than AMX under both conditions. Previous studies [36,37] reported that SDZ could only be biodegraded by 50%–60% after 11–290 h, therefore, its biodegradation in the MBR operation at short HRT of 4 h would be limited. The amount of SDZ adsorbed onto sludge particles was found increasing with time. Furthermore, E/S ratio of 0.89–0.91 in all conditions illustrated that the membrane filtration played only partial role in the SDZ rejection. Similar observation of ineffective retention of SDZ by membrane filtration was also reported [38]. However, the accumulation of SDZ up to 100 μg L^{-1} in the MBR did not yield adverse impact on organic and nitrogen removal because its residual concentration was much lower than the reported critical level of 6 mg L^{-1} [39].

For more hydrophilic compound, i.e., TMP ($\log K_{ow}$ = 0.9), biodegradation was found to be the predominant mechanisms in its removal while adsorption onto the suspended sludge was observed at low level. There was no significant difference ($p > 0.05$) in the TMP removal under no sludge wastage and constant sludge concentration (5 g L^{-1}) conditions. The major part of TMP removal came from its biodegradation as indicated by low S/I ratio of 0.1–0.15. Meanwhile, low E/S ratio of 0.4–0.41 also suggests that membrane filtration played a significant role in retaining untreated TMP within MBR. Most untreated TMP may be firstly retained in the supernatant and then removed through microbial degradation.

3.3. Antibiotic removal mechanisms

The antibiotic removal mechanisms through adsorption and biodegradation by the aerobic sludge are presented in Fig. 3 whereas the first-order rate constant (K_{bio}) of the studied compounds with their coefficient of determination (R^2) are shown in Table 3. For AMX, batch experiment results revealed that biodegradation was the main mechanism in this MBR. The low adsorption of AMX took place during initial period (Fig. 3a). Afterward, biodegradation was found to be the dominant removal mechanism, with first-order biodegradation rate constant of 0.43 h^{-1} while maximum adsorption capacity of MBR sludge

Table 2
Average antibiotic concentrations and their removal in the MBR

Compound	No sludge wastage condition (n = 8)			Constant sludge of 5 g L^{-1} (n = 8)		
	Influent	Effluent	Removal %	Influent	Effluent	Removal %
AMX (μg L^{-1})	73.2 ± 26.6	<0.1	100	62.3 ± 33.4	0.1 ± 0.1	99.8
SDZ (μg L^{-1})	116.5 ± 12.7	66.7 ± 18.2	42.6	121.8 ± 18.8	72.2 ± 9.2	36.4
TMP (μg L^{-1})	102.9 ± 35.3	3.5 ± 3.0	95.6	118 ± 20.2	6.7 ± 3.0	94.5

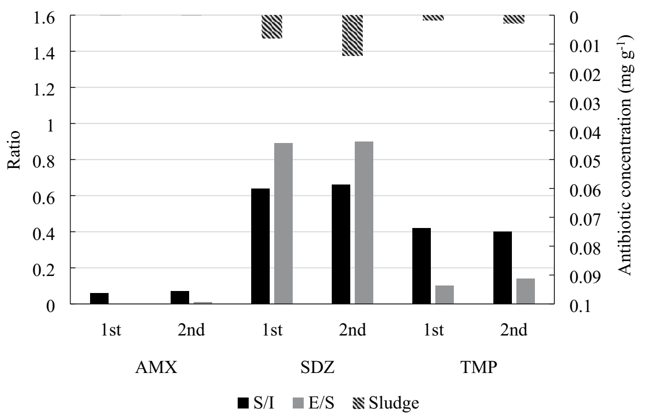

Fig. 2. Concentration ratios of supernatant to influent (S/I) and effluent to supernatant (E/S) and adsorbed antibiotics on MBR sludge.

for AMX was determined at 0.10 mg g^{-1} MLSS. Initial adsorption onto MBR sludge followed by fast hydrolysis reaction during microbial degradation possibly contributed to AMX removal. Due to high biodegradation rate of AMX of MBR sludge, there was no observed difference in the AMX removal, when the MBR was operated even under lower sludge concentration of 5 g L^{-1}.

On the contrary, the removal of SDZ by aerobic sludge was mostly occurred through adsorption with insignificant biodegradation (Fig. 3b). The batch experiment revealed that the first-order biodegradation rate constant of SDZ was as low as 0.001 h^{-1}, several orders less than that of AMX. Nevertheless, the adsorption capacity of SDZ (0.29 mg g^{-1} MLSS) was almost three times higher than that of AMX. Therefore, the untreated SDZ was largely presented in supernatant. During the experiment over a year, the accumulation of SDZ in MBR sludge was found only at 4.8% of its maximum adsorption capacity therefore sustainable operation could be expected over long-term operation. Previous research [36] illustrated that aqueous concentration of SDZ slightly decreased with the increased mass fraction of 11.5%–12% during 48 h while approximately 50% of SDZ could be degraded by pure microbial culture after 11 h. Moreover, sulfonamide antibiotics need a lag period of 6–12 d before they were removed in acclimated sludge through first-order or zero-order degradation kinetics [41]. Therefore, under typical treatment time of 6 h in activated sludge system, SDZ removal by only 2 μg L^{-1} was observed [41].

Meanwhile, the biodegradation also played as significant role in TMP removal while adsorption was found as supportive mechanism (Fig. 3c), with maximum adsorption capacity of 0.08 mg g^{-1} MLSS. The first-order biodegradation rate constant of TMP was determined as 0.033 h^{-1}, which was about 12 times slower than that of AMX. Nevertheless, the TMP biodegradation rate was much faster than that of SDZ. Moderated biodegradation of TMP under the aerobic condition could contribute from the enzyme produced from biomass cultivated in the MBR operated under long SRT condition. The increase TMP removal with increasing sludge age condition was reported

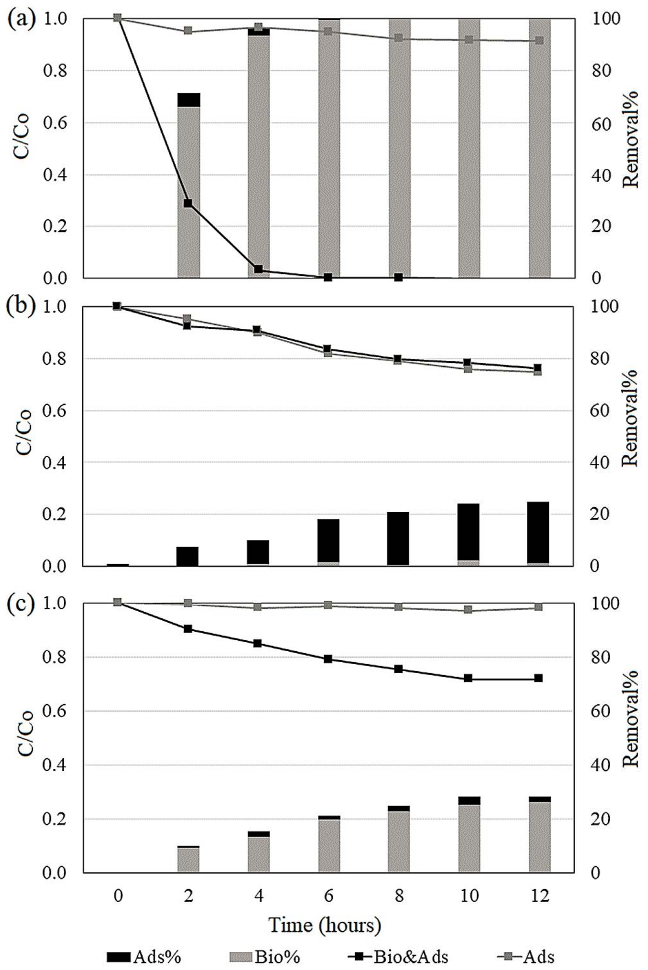

Fig. 3. Removal of antibiotics through adsorption and biodegradation by MBR sludge. (a) Amoxicillin, (b) sulfadiazine and (c) trimethoprim.

Table 3
First-order biodegradation rate constant (K_{bio}) of antibiotics by MBR sludge

Antibiotics	First-order biodegradation rate constant K_{bio} (h^{-1})	Coefficient of determination (R^2)
AMX	0.47	0.94
SDZ	0.001	0.96
TMP	0.033	0.97

in the previous research [42]. Moreover, the combination of high SRT and low sludge loading could lead to increase in biodiversity of activated sludge, which seen to contribute a greater impact on the elimination of antibiotic undergoing co-metabolism as the TMP was removed by nitrifying bacteria like other nitrogenous organic compounds [13,43,44]. The degradation of TMP to NH_4^+, and then from NH_4^+ to NO_3^- in a continuous aerobic nitrification process was also reported [13]. During the MBR operation, TMP was found accumulated up to 3.5% of its maximum adsorption capacity. This low adsorption level of TMP was possibly attributed from its low adsorption rate onto sludge as well as higher biodegradation rate.

In overall, AMX and TMP with hydrophilic nature were mainly biodegraded in the MBR. In contrast, SDZ was mainly removed through adsorption with low biodegradation. Most untreated SDZ were largely presented in the supernatant and mostly left the MBR. The operation of MBR at high sludge concentration and longer HRT would yield better removal of this antibiotic. Nevertheless, MBR operated at short HRT of 4 h could not completely remove recalcitrant antibiotic such as SDZ.

4. Conclusion

The MBR operated under short HRT of 4 h yielded high BOD and TKN removal (>85%) from synthetic aquaculture farm wastewater containing antibiotics. The incorporation of sponge media in MBR improved TN removal through promoting denitrification in the aerobic reactor. The removal of studied antibiotics varied from 36% for SDZ to >99% for SMX depending on their properties and removal mechanisms. While AMX and TMP were highly biodegraded, and SDZ was left largely presenting in the supernatant of mixed liquor. AMX was rapidly biodegraded whereas TMP and SDZ were much slowly removed. Nevertheless, TMP could be highly retained by microfiltration membrane resulting good overall removal efficiencies whereas SDZ was only partially removed. The operation of MBR at higher biomass concentration in MBR could improve the removal of antibiotics but post-treatment would be required for further removal of recalcitrant compounds such as SDZ.

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