Effect of Ciprofloxacin dosages on the performance of sponge membrane bioreactor treating hospital wastewater

Thanh-Tin Nguyen[1], Xuan-Thanh Bui[2,*], Bao-Trong Dang[3], Huu-Hao Ngo[4], Deokjin Jahng[5], Takahiro Fujioka[6], Shiao-Shing Chen[7], Quoc-Tuc Dinh[8], Cong-Nguyen Nguyen[9], Phan-Thai-Vy Nguyen[2]

[1]*Environmental Engineering and Management Research Group & Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Vietnam, email:* nguyenthanhtin@tdt.edu.vn.

[2]*Faculty of Environment and Natural Resource, Ho Chi Minh City University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam. Email:* bxthanh@hcmut.edu.vn

[3]*Institute of Research and Development, Duy Tan University, 03 Quang Trung, Da Nang, Vietnam.*

[4]*Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Broadway, NSW 2007, Australia.*

[5]*Department of Environmental Engineering and Energy, Myongji University, Republic of Korea.*

[6]*Graduate School of Engineering, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.*

[7]*Institute of Environmental Engineering and Management, National Taipei University of Technology, Taipei 10608, Taiwan.*

[8]*NTT Institute of Hi-Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.*

[9]*Faculty of Environment and Natural Resources, Da Lat University, Vietnam*

Abstract

This study aimed to evaluate treatment performance and membrane fouling of a lab-scale Sponge-MBR under the added Ciprofloxacin (CIP) dosages (20; 50; 100 and 200 μg L^{-1}) treating hospital wastewater. The results showed that Sponge-MBR exhibited effective removal of COD (94-98%) during the operation period despite increment of CIP concentrations from 20 to 200 μg L^{-1}. The applied CIP dosage of 200 μg L^{-1} caused an inhibition of microorganisms in sponges, i.e. significant reduction of the attached biomass and a decrease in the size of suspended flocs. Moreover, this led to deteriorating the denitrification rate to 3-12% compared to 35% at the other lower CIP dosages. Importantly, Sponge-MBR reinforced the stability of CIP removal at various added CIP dosages (permeate of below 13 μg L^{-1}). Additionally, the fouling rate at CIP dosage of 200 μg L^{-1} was 30.6 times lower compared to the control condition (no added CIP dosage).

Keywords: Ciprofloxacin; dosage; sponge membrane bioreactor; hospital wastewater.

1. Introduction

In recent years, the occurrence of antimicrobials has been emerged as a critical problem due to their risk of causing the undesirable ecosystem and human health (Kümmerer, 2009). Antimicrobials, namely antibiotics, are one of the most important drugs to prevent and treat infectious diseases (Tran et al., 2016a). These substances were presented at various concentrations in the aquatic environment in Vietnam (i.e., sulfamethoxazole (2.5 ± 1.9 μg L^{-1}), norfloxacin (9.6 ± 9.8 μg L^{-1}), ciprofloxacin (5.3 ± 4.8 μg L^{-1}), ofloxacin (10.9 ± 8.1 μg L^{-1}), erythromycin (1.2 ± 1.2 μg L^{-1}), tetracycline (0.1 ± 0.0 μg L^{-1}), and trimethoprim (1.0 ± 0.9 μg L^{-1}) (Vo et al., 2016). Most wastewater treatment plants (WWTPs) were not intentionally designed for antibiotic removal (Luo et al., 2014). Especially, there were many antibiotic groups such as Sulfonamide, Fluoroquinolone and Macrolide found in real hospital wastewater. The most widely prescribed fluoroquinolone antibiotic is ciprofloxacin (CIP) (Santos et al., 2013). For instance, CIP was found in hospital wastewaters in Viet Nam, with the concentrations being 4-53 times higher than those in other Asian countries such as China and Australia (Vo et al., 2016). As well known, a recent study indicated that CIP was one of the major antibiotics registered for production in the 5-year (2008-2013) in Viet Nam (Thai et al., 2018). Their investigation reported that the high concentration of CIP (41 μg L^{-1}) was detected from the hospital wastewater. Another one, some studies showed that CIP was detected at the different concentration ranges, namely 7.9 – 87.3 μg L^{-1} (Lien et al., 2016), 1.1 – 44 μg L^{-1} in Viet Nam (Duong et al., 2008), 3.6-101 μg L^{-1} in Sweden (Lindberg et al., 2007), 0.7 – 125 μg L^{-1} in Germany (Hartmann et al., 1998), and 32-99 μg L^{-1} in Brazil (Martins et al., 2008). Regarding the CIP removal, it has been previously reported with poor degradation of 32% after 48 h corresponded with the influent of 96.7 μg L^{-1} (Li and Zhang, 2010). Another study indicated that despite a low concentration of 2.2 μg L^{-1} in feed water the CIP removal was still negative when applied the conventional activated sludge (CAS) (Blair et al., 2015).

Membrane bioreactors (MBR) has been widely known as a potential technology to advance the water sustainability. The MBR system is a combination of a suspended growth in a bioreactor with a filtration on a porous membrane unit, which allows an operation at high biomass retention, microbial diversity and brings a promise for the degradation of micro-pollutants thus improving treated water quality (Cheng et al., 2018). Compared to CAS process, MBR exhibited an enhanced elimination of several pharmaceutical residues e.g., Sulfonamides, Macrolides, Tetracyclines, Indomethacin, Diclofenac, Propyphenazone, Pravastatin and Gemfibrozil (Radjenović et al., 2009). The long sludge age maintained in MBR helps to improve the removal of slowly degradable antibiotics. Furthermore, the longer sludge retention time (SRT) favored for the growth of nitrifying bacteria which helps to enhance degradation of the antibiotics (e.g. Ofloxacin, Sulfamethoxazole, Trimethoprim, Erythromycin, Roxithromycin) (Tran et al., 2016). In literature, the CIP removal was investigated at different feed concentration as well as various technologies used from the previous works. In detail, conventional MBR, anoxic-oxic MBR and aerobic granular sludge MBR showed efficiency about 51±13 % (Kovalova et al., 2012); 58.6 ± 6.2% (Hamjinda et al., 2017) and 15.8 ± 5.1% (Zhao et al., 2014), corresponding with the feed concentration of 2.33 - 3.75 μg L^{-1}, 17.92 - 46.04 μg L^{-1} and 50.29 - 54.19 μg L^{-1}, respectively. Kim et al. (2014) studied on the real wastewater with the CIP concentration of 1.2-1.38 μg L^{-1} based on the MBR process; their findings indicated that the CIP removal referred to the strong sorption to sludge (i.e., 98 % removal). Another one, Dorival-García et al. (2013) reported that an improvement of the CIP biodegradation efficiency could obtain about 52.8% in the MBR system when operating the appropriate conditions i.e., SRT of 30 days, the temperature of 38^0C and MLSS of 15000 mg L^{-1}. Another hand, for the key drawbacks of MBR application this system could not improve the total nitrogen removal due to a lack of anoxic zone whilst the membrane fouling became a crucial challenging for the wide practical application. Clearly, membrane fouling in MBR is a major obstacle that needs to be resolved (Chen et al., 2016; Teng et al., 2018). A study of Shi et al. (2017) reviewed the important role of extracellular polymeric substances (EPS) in controlling fouling in the MBR process. Their findings showed that concentration and characteristic of EPS are two vital factors that determine the degree and severity of fouling condition. Meanwhile, membrane fouling with the presence of pharmaceuticals/antibiotics has been paid to attention to a topic of discussion recently for understanding and the fouling control. The past studies revealed that the presence of pharmaceutical products caused an effect on the membrane fouling by inducing the microbial effects on activated sludge or changing the character of soluble microbial products (SMPs) and extracellular polymeric substances (EPS) (Li et al., 2015). However, for particular CIP a little attention has been directed to its role in influencing membrane fouling of the MBR process. A study of (Meng et al., 2012) indicated a role of certain CIP on the fouling propensity in the MBR. Their findings indicated that the fouling rate exposed the CIP of 1000 μg L^{-1} was much lower compared to that of MBR control, i.e. the absence of CIP, thereby making a positive role in MBR fouling control.

To enhance the nitrogen removal as well as the fouling control, sponge carrier, thus, has been introduced as an ideal attached growth media coupling with the MBR process (Ngo et al., 2006). As well known, sponge-MBR was demonstrated with less fouling rather than the conventional MBR i.e. 10-40 times reduction (Nguyen et al., 2016). Another one, sponge-MBR, a strong candidate, gave a better alternative to conventional MBR with an enhancement of total nitrogen removal (Thanh et al., 2013). For antibiotic removal, Nguyen et al. (2017) recently indicated that the CIP removal in Sponge-MBR (i.e., hollow fiber membrane module) reached 70% treating real hospital wastewater i.e. low CIP concentration of 23.84 μg L^{-1}. As mentioned beforehand, however, the concentration of CIP in wastewater was dependent on area, i.e., urban and rural or even country (125 μg L^{-1} in Germany). For example, Lien et al. (2016) reported the highest CIP concentration was 40.4 μg L^{-1} and 87.3 μg L^{-1} detected at the urban hospitals and the rural hospitals in Vietnam, respectively. Although Sponge-MBR has brought the positive removal and the fouling reduction when it was investigated with real hospital wastewater, i.e. low CIP concentration, in some cases CIP was detected with the high concentration as aforementioned. Moreover, it is noted that a systematic investigation of Sponge-MBR under different CIP gradients has not been sufficiently reported in the literature yet; therefore this need to be clarified. In this study, as a low CIP concentration of 12.85 ± 10.9 μg L^{-1} in hospital wastewater was studied, we added different CIP dosages (20; 50; 100; 200 μg L^{-1}) to evaluate the change of biomass (in sponge carrier via SEM visualization, suspended sludge via particle size distribution (PSD)), the performance (COD, nitrogen, CIP removal) and the fouling propensity (correlation between PSD and TMP data). To be the best our knowledge, this is a first research to clearly make that point.

2. Materials and method

2.1. Hospital wastewater and seed sludge

Hospital wastewater taken from a hospital in Ho Chi Minh City (HCMC) was used for this study. The concentration of wastewater is in mg L^{-1} (physical-chemical parameters) and μg L^{-1} (concentration of CIP), with COD (402±155), TKN (23±9), NH_4^+-N (7.4±3.2), TP (0.6±0.3) and CIP (12.85±10.9). The seed sludge from a conventional MBR system was used to acclimatize for the sponge-MBR, reaching to 5000 mg VSS L^{-1}. The ratio of MLVSS/MLSS of this seed sludge is 0.7.

2.2 Stock solutions of CIP

CIP stock solutions 500 mL was prepared to obtain the concentration of CIP of 3.6 g L^{-1} by dissolving CIP in methanol. Except for the control condition, i.e. no added CIP dosages, the stock solution was kept at 4◦C before introducing into raw wastewater to reach the added dosage of 20, 50, 100 and 200 µg L^{-1}, respectively.

2.3 Operating conditions of Sponge-MBR

In this study, a tank with the volume of 100 L was used for feeding into MBR reactor (denoted at Fig. 1). After 3 days of operation, a fresh hospital wastewater with the desired spiking CIP concentrations (20; 50; 100; 200 µg L^{-1}) was prepared. A glass reactor with a working volume of 8 L and the dimension of L×W×H = 0.28m × 0.08m × 0.6m was used for the lab-scale. A hollow fiber membrane module (Mitsubishi Rayon, Japan) with the membrane surface area of 0.05 m^2 and the pore size of 0.4 µm was submerged in the reactor. Polyurethane sponge carrier (APG, Japan), with the density of 30 kg m^{-3}, the porosity of 98% and the specific surface area of 3000 m^2 m^{-3}, was used as the moving carries. An optimized sponge cube of 1 cm^3 (dimension of 1cm x 1cm x 1cm) was selected to introduce into the reactor with the occupation of 20% (v/v) reactor volume. The sponge-MBR was automatically operated by using timers, solenoid valves, and a digital pressure gauge. The system was maintained at intermittent suction cycles with a filtration time of 8 mins and a relaxation time of 2 mins. A diffused aeration system was used to supply oxygen for the microbial growth and the membrane scouring. The Sponge-MBR was operated with a high flux of 20 LMH**,** corresponding to organic loading rate (OLR) of 1.12 ± 0.26 kg COD $m^{-3}d^{-1}$. The trans-membrane pressure (TMP) was measured by a digital pressure gauge, used as an indicator of the membrane fouling propensity. Chemical cleaning for membrane was conducted when TMP reached 50 kPa. The membrane was firstly washed with tap water to remove the cake layer on the membrane surface before immersed in sodium hypochlorite (concentration of 3000 mg L^{-1}) and sodium hydroxide (4% v/v) for 8 h. For the experimental conditions, the stock solution was kept at 4◦C before introducing into raw wastewater to reach the added CIP dosage of 20, 50, 100 and 200 µg L^{-1}, respectively. Particularly, for the operating conditions, the Sponge-MBR maintained 8 h for hydraulic retention time (HRT) and 20 days for suspended sludge retention time (SRT). No sponges were taken out of reactor except the tiny debris from broken sponges. To control the SRT of 20 days, the volume of waste sludge (suspended biomass applied for the practical application) was 0.4 L d^{-1}. This operation was to preserve the sponges and to retain slow-growing bacteria in the reactor. For this operation, the "real SRT" maintained in Sponge-MBR was slightly higher than the "control SRT", i.e. 20 days, because there was a certain amount of biomass in the sponges which was always retained in the reactor.

2.3. Analytical methods

2.3.1 Physical-chemical parameters

Parameters such as COD, TKN, NH_4^+-N, NO_2^--N, NO_3^-—N, and TP were analyzed according to Standard Methods (APHA, 2005). A protocol for determining the biomass attached in the sponge was adapted from the previous work (Escolà Casas et al., 2015). To determine the sludge concentration, the attached biomass in sponge carriers was converted into mixed liquor suspended solids (MLSS) concentration. Ten sponge carriers from MBR reactor placed in the aluminum foil cups were dried overnight at 105 °C, followed by weighing. For the biomass detachment in sponges, these carriers were washed in a solution of 2 M NaOH and cleaned with de-ionized water. Afterward, they were dried at 105 °C overnight and weighed again. The biomass on the carriers was determined based on the weight difference before and after cleaning of the carriers.

2.3.2 Sludge characterization

Original and operating sponges were observed by Field-emission Scanning Electron Microscope (Hitachi's S-4800 FE-SEM). Initially, a pretreatment of sponges for SEM observation was performed by Upright Ultra-Low Temperature Freezers (Sanyo MDFU72VC, Japan) at -50ºC for 3 h. Then, dehydration was conducted throughout the freeze-drying process (Eyela FDU-2100, Tokyo Rikakikai Co. Ltd., Japan) for 24 h. The trap cooling temperature was automatically controlled at -80ºC. The solid phase of water in the sample will directly transfer to the vapor phase using Oil Filtration Vacuum Pump (GCD-136XNF) operated at the pressure of 0.067 kPa. This method prevents the migration of nonvolatile molecules, therefore retaining of form and dimension of the structure. Lastly, the samples were coated with platinum (Pt) layer of approximately 5 nm thickness by an ion sputter coater (Hitachi E-1045, Japan) and imaged using an SEM detector with accelerating voltage of 5 kV. In addition, to determine the size of suspended sludge, a particle size distribution (PSD) analysis was performed using a Laser Scattering Particle Analyzer (Horiba LA-950, Japan). The measured particle size was ranged from 0.01 – 3000 μm.

2.3.3 Determination of membrane resistances

The membrane resistances were determined based on our previous work (Nguyen et al., 2016). The calculation was employed with the Darcy equation (Eq. 1 and Eq. 2). In brief, recording flux (J) and TMP (ΔP) were used to determine the resistance based on Eq. 1 and Eq. 2. After TMP reached 50 kPa, the membrane module was taken out to filter with the pure water, which was used to determine the total resistance (R_t). The cake resistance (R_c) regarded as deposition of the cake layer onto membrane surface that can be completely flushing using tap water. Thus, the total of (R_f+R_m) can be defined by the filtration of pure water with removing the cake layer. Subsequently, R_c can be calculated by subtraction of the total resistance (R_t) and the total of (R_f+R_m). After that, the membrane was soaked in the cleaning agents of 0.5% NaOCl and 4% NaOH for 4 hours to determine the membrane resistance. Finally, R_f is determined using Eq. 2.

$$J = \Delta P/(\mu . R_t) \qquad \text{(Eq. 1)}$$

$$R_t = R_m + R_c + R_f \qquad \text{(Eq. 2)}$$

Where J is the permeate flux; ΔP is trans-membrane pressure (TMP); μ is the viscosity of permeate; R_t is the total resistance; R_m is the intrinsic membrane resistance; R_c is the cake layer resistance and R_f is the fouling resistance caused by the adsorption of soluble matters and the pore blocking.

2.3.4 Analysis of target CIP

For sample preparation, 50 mL water samples were firstly filtrated by 0.45 μm membrane filter. Regarding the concentrated CIP concentration, Oasis® hydrophilic-lipophilic - balance (HLB) cartridges (60 mg, 3 mL, Waters, Corp., Milford, MA) were used for the solid phase extraction (SPE) based on the method of previous study (Dinh et al., 2011). Firstly, the cartridges were activated by using 3 mL of MeOH, 3 mL of ultrapure water. The samples were injected to the cartridges with the flow rate of 2-3 mL min^{-1}, followed by rinsing again with 3 mL of the ultrapure water/MeOH (90:5, v/v). The cartridges were then dried under the vacuum condition for 10 min to remove water. After that, the tested tubes were used to load CIP antibiotic which was eluted using 6 mL of methanol. The extracts were evaporated under N_2 and dissolved in 0.5 mL of a mixture of water/methanol ratio (90/10 v/v) and formic acid (0.01%), corresponded to the mobile phase of the LC-MS/MS. Finally, they were then passed through 0.2 μm syringe filters and stored in the vials before analyzing by Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). An LC-MS/MS system (Agilent1200 series) equipped with an Agilent Zorbax Eclipse Plus C18 column (with diameter, length, and pore size of 2.1 mm, 150 mm, 3.5 μm, respectively) was used to measure the concentration of antibiotic in the feed and permeate.

3. Results and discussion

3.1 Biomass fraction in Sponge-MBR under different CIP dosages

Fig. 2 shows the change of sludge fraction (attached (sponge) and suspended biomass concentration) in Sponge-MBR with the adding increment of CIP dosages (20; 50; 100; 200 μg L^{-1}). For the control condition, i.e. no added CIP dosages, MLVSS concentration of the attached biomass and the suspended biomass were 2758 mg L^{-1} and 2470 mg L^{-1}, respectively whilst the average ratio of the attached biomass/total biomass was 0.53 after 55 days operation. Nevertheless, for the added CIP dosage of 20 μg L^{-1} a decrease in total biomass was observed when operated for 33 days operation, considerably reduced to 1959 mg L^{-1} of the attached biomass and 1472 mg L^{-1} of the suspended biomass, in that order. It is noted that the mild higher ratio of the attached biomass/total biomass ratio (0.57) was indicated. This was attributed to a significant reduction in total biomass concentration (from 5228 mg L^{-1} to 3431 mg L^{-1}). For a consecutive increase of the CIP dosages, i.e. 50, 100, 200 μg L^{-1}, a strong effect of CIP on the attached biomass was noted when the ratio of the attached biomass/total biomass significantly reduced to 0.48, 0.4, and 0.33, respectively. Interestingly, although organic loading rate (OLR) increased from 1.3 to 1.7 kg COD m^{-3} d^{-1} which resulted in increasing the suspended biomass (from 2217 to 2802 mg L^{-1}), the attached biomass went down significantly, i.e. from 2115 to 1351 mg L^{-1} when the added high CIP was increased from 50 to 200 µg L^{-1}. These results indicated for the high CIP dosage, i.e. 100 and 200 µg L^{-1}, it led a negative impact on the attached biomass, thereby induced biofilm detachment which was observed by SEM (Supplementary data). The high CIP concentration caused a biological toxicity to microorganisms in the anoxic zone which were reported from the previous studies such as 24-40 mg L^{-1} (Liu et al., 2013a), 0.9 and 9 mg L^{-1} (Meng et al., 2015). A scanning electron microscope (SEM) observation of the attached biomass was conducted in this study to clearly make the effect of the added CIP dosages (Supplementary data). Those photos indicated that the frequency of the protozoa presence diminished as following order: no added CIP dosage, at the dosages of 20, 50, 100 µg L^{-1}. Whilst for the high dosage of 200 µg L^{-1} there was the absence of protozoa. As well known the protozoa is a good indicator for the activated sludge and plays an important role in the sludge's biological balance. For the SRT of 20 days retained in Sponge-MBR, in this study, much more protozoa (i.e., s*talk ciliates*) were observed within the sponges under the control condition, i.e. no the added CIP dosage. As observed, it was clear that protozoa well grew in the high porosity of sponge carriers. Furthermore, much biofilm inside the sponge carriers could be a good condition for simultaneous nitrification and denitrification (SND), yielded total nitrogen removal effectively (Thanh et al., 2013; Nguyen et al., 2017).

Insert Figure 2

3.2. Effects of CIP dosages on the treatment performance of Sponge-MBR

3.2.1 Organic removal

Insert Table 1

Table 1 presents the average COD concentration and the removal efficiency when increasing the added CIP dosages. Although the initial COD ranged from 344 - 467 mg L^{-1}, the average COD concentrations in permeate were always as low as 11-22 mg L^{-1}. Whilst the removal efficiency was from 94 to 98% for all conditions. Under the control condition i.e. no added CIP dosage, the removal efficiency of 96% was agreed with a study of Nguyen et al. (2017). Nevertheless, as spiking the CIP dosage of 20 μg L^{-1} the system was immediately impacted by a significant reduction in total biomass concentration (as mentioned in section 3.1); thereby causing a mild decrease of the removal efficiency (only 94%) and a slight increase of permeate (22 mg L^{-1}), which was likely contributed by the cell debris (Başaran et al., 2014). Whilst for the added dosage of 50 μg L^{-1} the removal was 98%. This was attributed to a recovery of total biomass as mentioned in section 3.1. As the results, the effect of CIP on the COD removal could be found when it was added the dosages of 100, 200 μg L^{-1}, which caused the increase of COD concentration in permeate i.e. 13, 22 mg L^{-1} respectively. Meng et al. (2015) reported that the higher COD concentrations of 47 – 69 mg L^{-1} in permeate were recorded under the pressure of high fluoroquinolones ranged from 0.9 – 9.0 mg L^{-1}, operated in an anoxic MBR system using synthetic wastewater. Their findings indicated that exposure to the fluoroquinolone, i.e., CIP could lead to temporary inhibition of bacteria responsible for COD removal. This was attributed that the fluoroquinolone inhibited aerobic Gram-positive and Gram-negative and some anaerobic Gram-negative species (Kohanski et al., 2010). Another one, Arya et al. (2016) demonstrated that only 70% TOC degradation was obtained in the presence of CIP compared its absence in anoxic MBR process (97%). Obviously, although the Sponge-MBR system was affected by high dosages of CIP, it allowed recovering the adaptation of microorganisms in long-term operation. This fact implies that the attached growth process in Sponge-MBR system could provide an opportunity for slow-growing microorganisms, allowing a longer acclimatization time of the microorganisms to the toxic compounds.

3.2.2 Nitrogen removal

Insert Table 2 and Table 3

Table 2 and Table 3 summarize the average concentrations of TKN, NH_4^+-N, NO_3^--N, NO_2^--N and TN in the feed and the permeate respectively. As denoted the permeate concentrations of NH_4^+-N and NO_3^--N meet the standard limits of the Vietnam National Technical Regulation on Health Care Wastewater (QCVN 28:2010/BTNMT) regulated with 10 mg NH_4^+-N L^{-1} and 30 mg NO_3-N L^{-1}. The removal efficiencies of total nitrogen (TN) were approximately 23 ± 20%; 29 ± 24%; 43 ± 32% and 41 ± 27% for the added CIP dosages of 20, 50, 100 and 200 μg L^{-1}, respectively. Meanwhile, a similar sponge-MBR applied by Nguyen et al. (2017) showed 53 ± 16% of TN removal without the addition of CIP dosage. This fact suggests that the TN removal was influenced by spiking the CIP dosages. The simultaneous nitrification and denitrification process (SND) has been well known as the incredible condition for the complete nitrogen removal, where autotrophic bacteria and the heterotrophic bacteria could grow under a gradient of dissolved oxygen (Deng et al., 2016). The past studies demonstrated that a supply of the sponge carriers in the MBR effectively enhanced the SND process (Thanh et al., 2013). In this case, the sponges yielded the proper condition for the attached biomass; thereby creating both aerobic and anoxic conditions (Thanh et al., 2013; Nguyen et al., 2017). Fig. 2 presents the nitrogen balance to validate the relationship between the nitrogen assimilation and the nitrogen denitrification for nitrogen removal. As the results, the nitrification rate was 0.14 mg NH_4^+-N $gVSS^{-1}$ day^{-1} at the control condition i.e. no added CIP dosage whilst for the added CIP dosage of 20 μg L^{-1} it decreased to 0.1 mg NH_4^+-N $gVSS^{-1}$ day^{-1}. It was noted that as spiking the CIP dosages in sponge-MBR it promoted the nitrification rate from 0.12-0.21 mg NH_4^+-N $gVSS^{-1}$ day^{-1} corresponded with the increment of 50-200 μg L^{-1}. This was attributed to that the presence of high CIP concentration (200-2000 μg L^{-1}) caused an increase of the proportion of ammonia-oxidizing bacteria (AOB) in the MBR system from 3.4% (the control condition, i.e., no added CIP dosage) to 5.5 % (the added CIP dosage of 200 μg L^{-1}) and 10.6% (the added CIP dosage of 200¬2000 μg L^{-1}) Yi et al. (2017). Moreover, Meng et al. (2015) indicated that the AOB population was also affected by the added CIP dosage i.e. 900-9000 μg L^{-1} caused a shift of dominant AOB from the *N. communis* lineage (84%) to the *N. oligotropha* lineage (60%) in the MBR system. Evidently, the high CIP dosage induced the certain effects on the population of AOB in the MBR process. In this study, therefore, the sponge-MBR yielded a positive impact on the nitrification rate although the reduction of total biomass was noted in section 3.1.

Insert Figure 3

Constantly, the added CIP dosage strongly affected the denitrification rate in the sponge-MBR system. Fig. 3 showed that the efficiency was significantly reduced when the CIP dosages were spiked, ranged from 3-12 %, compared to 35 % of the control condition, i.e., no added CIP dosage. This was attributed to the detachment of biofilm from sponge carriers, causing significant loss of anoxic zone in the sponges where denitrifying microbes existed. The SEM photographs (Supplementary data) showed this effect. Likewise, a past study of Yi et al. (2017) applied a conventional process (SBR), their findings indicated that the denitrification process was influenced significantly when prolonged the exposure of CIP dosage, causing an increase of the effluent nitrite from 0.17 mg L^{-1} (the control condition, i.e., no added CIP dosage) to 1.66-4.55 mg L^{-1} (the added CIP dosage of 200-2000 µg L^{-1}). Another one, as reported in the study of Meng et al. (2015), an anoxic–aerobic MBR was operated at the SRT of 20 days and the hydraulic retention time (HRT) of 10 h when spiked the CIP dosage of 900-9000 µg L^{-1}. They reported that the decrease of specific denitrification rate (SDNR) from 12.7 to 8.9 mg g $MLVSS^{-1}$ h^{-1} was likely due to the inhibition of fluoroquinolones, i.e., CIP on the overall activity of denitrifiers. Additionally, Liu et al., (2013a) also showed that CIP caused an inhibition of denitrification by CIP gradually increased with the long-term incubation of the batch aerobic experiments. It is noted that for the added CIP dosage of 200 µg L^{-1} the sufficient TN removal in still remained in this study. This was attributed to the majority of TN assimilation (Fig. 3) and lower TN concentration in feed compared to the others (denoted in Table 2).

3.2.3 CIP removal in Sponge-MBR

Insert Figure 4

Biodegradation, chlorination, ozonation, photolysis, and adsorption processes have been recently introduced for the elimination of antibiotics from wastewaters (Sharma et al., 2017). It is certainly that CIP removal by either evaporation or photodegradation was less considerable in the Sponge-MBR system. In detail, the effect of photodegradation in the activated sludge processes was negligible since the high sludge concentrations limited the penetration of sunlight. In this study, therefore, the adsorption and biodegradation have been considered as major mechanisms for the CIP removal. Fig. 4 denoted that the CIP removal efficiency was ranged from 78% to 94% when operated at the added dosage of 20-200 μg L^{-1}. As the results, although the added CIP dosage was dramatically increased during operation (20; 50; 100; 200 μg L^{-1}), Sponge-MBR system still performed the sufficient removal, with the permeate concentrations ranged from 6.5-13 μg L^{-1}. As mentioned in section 3.1, the attached biomass was detached from sponge carriers and consequently released to the mixed liquor in the reactor. This fact, therefore, caused the gradual increase of the suspended biomass in Sponge-MBR system, which could help to retain a stable CIP removal. Liu et al. (2013c) demonstrated that the suspended microorganism could exhibit a tolerance with CIP exposure in prolonged incubation of aerobic condition and the adsorption was considered as the major mechanism. Another study of Girardi et al. (2011) indicated CIP was recalcitrant to biodegradation in the aqueous system. In addition, the previous study showed the effect of pH value on the characteristics of CIP. Thus with the neutral pH value retained in the Sponge-MBR, CIP would bring a structure of a carboxyl and an amine group. That structure gave the ability for the simultaneous occurrence of electrostatic force interactions and the divalent bridges to form adsorption mechanism (Conkle et al., 2010). By this way, neither the amine-positive charged nor the carboxyl group of CIP could interact with the negatively charged sludge (Dorival et al., 2013). Moreover, this was certainly confirmed that CIP could be significantly adsorbed onto solid with high K_d of 1500 L kg SS^{-1} (Sipma et al., 2010). The study of Dorival et al. (2013) showed that CIP could exhibit a high sorption onto the sludge in a conventional MBR (i.e., with K_d of 516-3746 L kg^{-1} corresponded to the temperature range of 9–38°C). Li and Zhang (2010) reported that a negligible biodegradation (<10%) of CIP was observed in the MBR process.

3.3. Effects of CIP dosages on fouling behavior

Insert Figure 5

Fig. 5 shows an increasing trend of the TMP values and the change of the sludge floc size for all added CIP dosages in the sponge-MBR system. As the results, there was a gradual increase in TMP under the control condition and the added CIP dosages of 20-50 μg L^{-1}. A chemical cleaning was conducted to reduce the fouling when the TMP value reached to 50 kPa at the end period. Constantly, when spiked the CIP dosages to 100 and 200 μg L^{-1}, the TMP value increased slightly and reached 18 kPa after 70 days operation. The fouling rate at this condition was relatively low with the value of 0.3 kPa d^{-1}. Interestingly, for the added CIP dosage of 200 μg L^{-1} the fouling rate was 30.6 times lower compared to the control condition (no added CIP dosage). This fact indicated that membrane fouling was considerably affected by the increment of CIP dosages. Moreover, the added CIP dosages caused the change of the sludge floc sizes; thereby making different membrane fouling behaviors between the conditions. As the results, the mean floc size of sludge in Sponge-MBR was 57 μm (the control condition, i.e., no added CIP dosage), 60 μm (20-50 μg L^{-1}), 54 μm (100 μg L^{-1}) and 27 μm (200 μg L^{-1}). Evidently, in this study when the added CIP dosage was higher 100 μg L^{-1} it caused a significant decrease of floc size in sponge-MBR. Meanwhile, Meng et al. (2015) reported that the floc size of sludge in the system considerably reduced by exposing a high CIP dosage of 900 μg L^{-1}. This was attributed due to the death of bacterial cells led to sludge deflocculation as well as the ease of biofilm detachment from the membrane module. Moreover, in this situation, the sponge carriers were in charge of function, with a lower density and a higher velocity leading to the stronger movement for chafing on the membrane surface; therefore, the floc size and membrane fouling significantly decreased.

Insert Table 4

Cake layer and pore blocking were indicated by the proportion of cake resistance (R_c) and fouling resistance (R_f) (Table 4). The fouling resistance of the fouled membrane was measured at the end of the experimental run. Total resistance (R_t) decreased 7.85×10^{12}; 7.15×10^{12} and 3.08×10^{12} m^{-1} respectively at the conditions: the control; 20-50 µg L^{-1}; 100-200 µg L^{-1}, which was consistent with the fouling trend as mentioned beforehand. The values of the R_C and R_f were $5.01x10^{12}$ and $2.51x10^{12}$; $5.01x10^{12}$ and $1.78x10^{12}$; $1.13x10^{12}$ and $1.52x10^{12}$ at the control condition; 20-50 µg L^{-1}; 100-200 µg L^{-1}, respectively. As the following order, the accounted percentage of 63.8% and 31.9%; 70.1.6% and 24.8%; 36.6% and 49.5% was calculated for the R_c and R_f. This showed that for the added CIP dosages of 100 and 200 µg L^{-1} the values of R_c and R_f were approximately 1.6 times lower than those of the control period. Normally, the original issues of membrane fouling are solutes and colloids from wastewater, in case of spiking the CIP into the reactor, the sludge structure was destroyed; thereby limiting the production of SMP and EPS which were major foulants (Meng et al., 2017). Therefore, for the Sponge-MBR system in this study, the added CIP dosage also exhibited the better membrane fouling mitigation by minimizing the adsorption and cake layer formation on membrane.

Conclusions

Firstly, organic removal was not remarkably affected by the added Ciprofloxacin (CIP) dosages up to 200 µg L^{-1}. Secondly, the added CIP dosage as higher as 20 µg L^{-1} significantly caused the biofilm detachment in sponge carriers as well as the decrease in size of suspended flocs. Thirdly, spiking the CIP dosage enhanced nitrification process whilst denitrification process was inhibited due to the decrease of attached biomass in sponge carriers. Finally, the added CIP dosages of 100-200 µg L^{-1} caused the reduction in membrane fouling via decrease in the resistances of fouling and cake layer.

Appendix: Supplementary data

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References

1. APHA, AWWA, WEF, 2005. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC
2. Arya, V., Philip, L., Murty Bhallamudi, S., 2016. Performance of suspended and attached growth bioreactors for the removal of cationic and anionic pharmaceuticals. Chem. Eng. J. 284, 1295–1307.

3. Başaran, S.T., Aysel, M., Kurt, H., Ergal, I., Akarsubaşi, A., Yağci, N., Doğruel, S., Çokgör, E.U., Keskinler, B., Sözen, S., Orhon, D., 2014. Kinetic characterization of acetate utilization and response of microbial population in super fast membrane bioreactor. J. Memb. Sci.
4. Blair, B., Nikolaus, A., Hedman, C., Klaper, R., Grundl, T., 2015. Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. Chemosphere 134, 395–401.
5. Chen, J., Zhang, M., Li, F., Qian, L., Lin, H., Yang, L., Wu, X., Zhou, X., He, Y., Liao, B.Q., 2016. Membrane fouling in a membrane bioreactor: High filtration resistance of gel layer and its underlying mechanism. Water Res.
6. Cheng, D.L., Ngo, H.H., Guo, W.S., Liu, Y.W., Zhou, J.L., Chang, S.W., Nguyen, D.D., Bui, X.T., Zhang, X.B., 2018. Bioprocessing for elimination antibiotics and hormones from swine wastewater. Sci. Total Environ. 621, 1664–1682.
7. Conkle, J.L., Lattao, C., White, J.R., Cook, R.L., 2010. Competitive sorption and desorption behavior for three fluoroquinolone antibiotics in a wastewater treatment wetland soil. Chemosphere 80, 1353–1359.
8. Deng, L., Guo, W., Ngo, H.H., Du, B., Wei, Q., Tran, N.H., Nguyen, N.C., Chen, S.S., Li, J., 2016. Effects of hydraulic retention time and bioflocculant addition on membrane fouling in a sponge-submerged membrane bioreactor. Bioresour. Technol. 210, 11–17.
9. Dinh, Q.T., Alliot, F., Moreau-Guigon, E., Eurin, J., Chevreuil, M., Labadie, P., 2011. Measurement of trace levels of antibiotics in river water using on-line enrichment and triple-quadrupole LC-MS/MS. Talanta 85, 1238–1245.
10. Dorival-García, N., Zafra-Gómez, A., Navalón, A., González, J., Vílchez, J.L., 2013.

Removal of quinolone antibiotics from wastewaters by sorption and biological degradation in laboratory-scale membrane bioreactors. Sci. Total Environ. 442, 317–328.

11. Duong, H.A., Pham, N.H., Nguyen, H.T., Hoang, T.T., Pham, H.V., Pham, V.C., Berg, M., Giger, W., Alder, A.C., 2008. Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam. Chemosphere 72, 968–973.
12. Escolà Casas, M., Chhetri, R.K., Ooi, G., Hansen, K.M.S., Litty, K., Christensson, M., Kragelund, C., Andersen, H.R., Bester, K., 2015. Biodegradation of pharmaceuticals in hospital wastewater by a hybrid biofilm and activated sludge system (Hybas). Sci. Total Environ. 530–531, 383–392.
13. Girardi, C., Greve, J., Lamshöft, M., Fetzer, I., Miltner, A., Schäffer, A., Kästner, M., 2011. Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. J. Hazard. Mater. 198, 22–30.
14. Hamjinda, N.S., Chiemchaisri, W., Chiemchaisri, C., 2017. Upgrading two-stage membrane bioreactor by bioaugmentation of Pseudomonas putida entrapment in PVA/SA gel beads in treatment of ciprofloxacin. Int. Biodeterior. Biodegrad. 119, 595–604.
15. Hartmann, A., Alder, A.C., Koller, T., Widmer, R.M., 1998. Identification of fluoroquinolone antibiotics as the main source of umuC genotixicity in native hospital wastewater. Environ. Toxicol. Chem. 7, 377-382.
16. Kim, M., Guerra, P., Shah, A., Parsa, M., Alaee, M., Smyth, S.A., 2014. Removal of pharmaceuticals and personal care products in a membrane bioreactor wastewater treatment plant. Water Sci. Technol. 69, 2221–2229.
17. Kohanski, M.A., Dwyer, D.J., Collins, J.J., 2010. How antibiotics kill bacteria: From targets to networks. Nat. Rev. Microbiol. 8 (6), 423-435.

18. Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., McArdell, C.S., 2012. Hospital Wastewater Treatment by Membrane Bioreactor: Performance and Efficiency for Organic Micropollutant Elimination. Environ. Sci. Technol. 46, 1536–1545.
19. Kümmerer, K., 2009. Antibiotics in the aquatic environment - A review - Part I. Chemosphere 75, 417–434.
20. Li, B., Zhang, T., 2010. Biodegradation and adsorption of antibiotics in the activated sludge process. Environ. Sci. Technol. 44, 3468–3473.
21. Li, C., Cabassud, C., Guigui, C., 2015. Evaluation of membrane bioreactor on removal of pharmaceutical micropollutants: a review. Desalin. Water Treat. 55, 845-858.
22. Lien, L.T.Q., Hoa, N.Q., Chuc, N.T.K., Thoa, N.T.M., Phuc, H.D., Diwan, V., Dat, N.T., Tamhankar, A.J., Lundborg, C.S., 2016. Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use-a one year study from Vietnam. Int. J. Environ. Res. Public Health 13, 1–13.
23. Lindberg, R.H., Björklund, K., Rendahl, P., Johansson, M.I., Tysklind, M., Andersson, B.A. V, 2007. Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Res. 41, 613-619.
24. Liu, Z., Sun, P., Pavlostathis, S.G., Zhou, X., Zhang, Y., 2013a. Inhibitory effects and biotransformation potential of ciprofloxacin under anoxic/anaerobic conditions. Bioresour. Technol. 150, 28–35.
25. Liu, Z., Sun, P., Pavlostathis, S.G., Zhou, X., Zhang, Y., 2013b. Inhibitory effects and biotransformation potential of ciprofloxacin under anoxic/anaerobic conditions. Bioresour. Technol. 150, 28–35.
26. Liu, Z., Sun, P., Pavlostathis, S.G., Zhou, X., Zhang, Y., 2013c. Adsorption, inhibition, and

biotransformation of ciprofloxacin under aerobic conditions. Bioresour. Technol. 144, 644–651.

27. Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641.
28. Martins, A.F., Vasconcelos, T.G., Henriques, D.M., Frank, C. da S., König, A., Kümmerer, K., 2008. Concentration of ciprofloxacin in Brazilian hospital effluent and preliminary risk assessment: A case study. Clean - Soil, Air, Water. 36, 264-269.
29. Meng, F., Gao, G., Yang, T.-T., Chen, X., Chao, Y., Na, G., Ge, L., Huang, L.-N., 2015. Effects of fluoroquinolone antibiotics on reactor performance and microbial community structure of a membrane bioreactor. Chem. Eng. J. 280, 448–458.
30. Meng, F., Wang, Z., Li, Y., 2012. Cure of filament-caused MBR fouling in the presence of antibiotics: Taking ciprofloxacin exposure as an example. Ind. Eng. Chem. Res. 51, 13784–13791.
31. Meng, F., Zhang, S., Oh, Y., Zhou, Z., Shin, H.-S., Chae, S.-R., 2017. Review Fouling in membrane bioreactors: An updated review. Water Res. 114, 151-180.
32. Ngo, H.H., Nguyen, M.C., Sangvikar, N.G., Hoang, T.T.L., Guo, W.S., 2006. Simple approaches towards the design of an attached-growth sponge bioreactor (AGSB) for wastewater treatment and reuse. Water Sci. Technol. 54, 191–197.
33. Nguyen, T.T., Bui, X.T., Luu, V.P., Nguyen, P.D., Guo, W., Ngo, H.H., 2017. Removal of antibiotics in sponge membrane bioreactors treating hospital wastewater: Comparison between hollow fiber and flat sheet membrane systems. Bioresour. Technol. 240, 42–49.
34. Nguyen, T.T., Bui, X.T., Vo, T.D.H., Nguyen, D.D., Nguyen, P.D., Do, H.L.C., Ngo, H.H.,

Guo, W., 2016. Performance and membrane fouling of two types of laboratory-scale submerged membrane bioreactors for hospital wastewater treatment at low flux condition. Sep. Purif. Technol. 165, 123–129.

35. Radjenović, J., Petrović, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43, 831–841.

36. Santos, L.H.M.L.M., Gros, M., Rodriguez-Mozaz, S., Delerue-Matos, C., Pena, A., Barceló, D., Montenegro, M.C.B.S.M., 2013. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. Sci. Total Environ. 461–462, 302–316.

37. Sipma, J., Osuna, B., Collado, N., Monclús, H., Ferrero, G., Comas, J., Rodriguez-Roda, I., 2010. Comparison of removal of pharmaceuticals in MBR and activated sludge systems. Desalination 250, 653–659.

38. Sharma, V., Kumar, R.V., Pakshirajan, K., Pugazhenthi, G., 2017. NU SC, Powder Technology. Elsevier B.V.

39. Shi, Y., Huang, J., Zeng, G., Gu, Y., Chen, Y., Hu, Y., Tang, B., Zhou, J., Yang, Y., Shi, L., 2017. Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: An overview. Chemosphere 180, 396–411.

40. Teng, J., Shen, L., Yu, G., Wang, F., Li, F., Zhou, X., He, Y., Lin, H., 2018. Mechanism analyses of high specific filtration resistance of gel and roles of gel elasticity related with membrane fouling in a membrane bioreactor. Bioresour. Technol.

41. Thai, P.K., Ky, L.X., Binh, V.N., Nhung, P.H., Nhan, P.T., Hieu, N.Q., Dang, N.T.T., Tam,

N.K.B., Anh, N.T.K., 2018. Occurrence of antibiotic residues and antibiotic-resistant bacteria in effluents of pharmaceutical manufacturers and other sources around Hanoi, Vietnam. Sci. Total Environ. 645, 393–400.

42. Thanh, B.X., Berg, H., Nguyen, L.N.T., Da, C.T., 2013. Effects of Hydraulic Retention Time on Organic and Nitrogen Removal in a Sponge-Membrane Bioreactor. Environ. Eng. Sci. 30, 194–199.
43. Tran, N.H., Chen, H., Reinhard, M., Mao, F., Gin, K.Y.H., 2016b. Occurrence and removal of multiple classes of antibiotics and antimicrobial agents in biological wastewater treatment processes. Water Res. 104, 461–472.
44. Vo, T.D.H., Bui, X.T., Cao, N.D.T., Luu, V.P., Nguyen, T.T., Dang, B.T., Thai, M.Q., Nguyen, D.D., Nguyen, T.S., Dinh, Q.T., Dao, T.S., 2016. Investigation of antibiotics in health care wastewater in Ho Chi Minh City, Vietnam. Environ. Monit. Assess. 188.
45. Yi, K., Wang, D., Li, X., Chen, H., Sun, J., An, H., Wang, L., Deng, Y., Liu, J., Zeng, G., 2017. Science of the Total Environment Effect of cipro fl oxacin on biological nitrogen and phosphorus removal from wastewater. Sci. Total Environ. 605–606, 368–375.
46. Zhao, X., Chen, Z. lin, Wang, X. chun, Shen, J. min, Xu, H., 2014. PPCPs removal by aerobic granular sludge membrane bioreactor. Appl. Microbiol. Biotechnol. 98, 9843–9848.

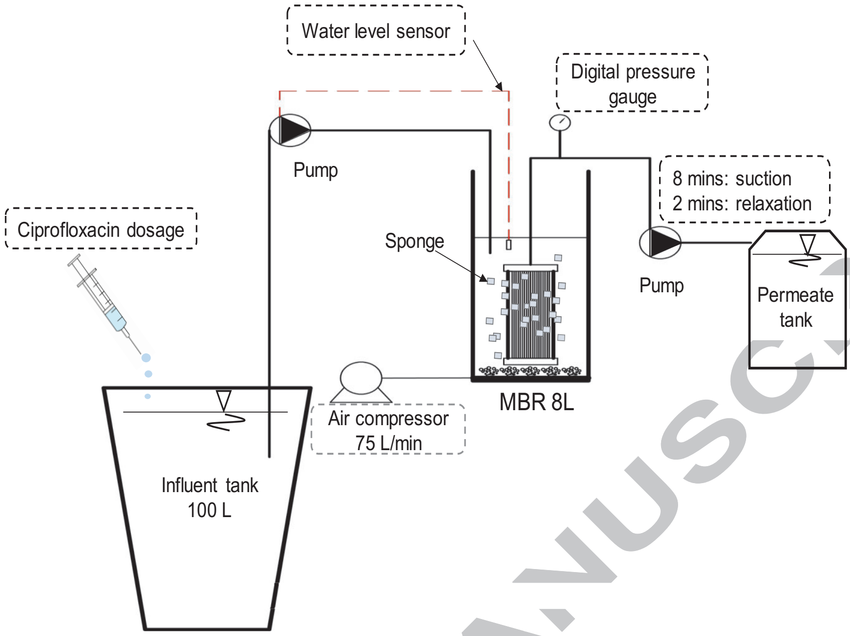

Figure 1. Schematic diagram of the lab-scale Sponge-MBR

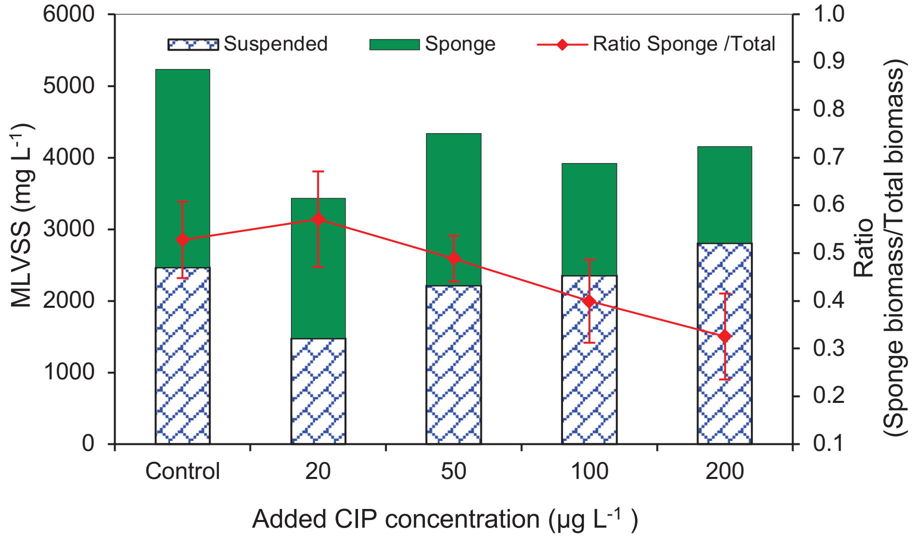

Figure 2. Effect of CIP dosages on suspended biomass and sponge (attached) biomass in Sponge-MBR

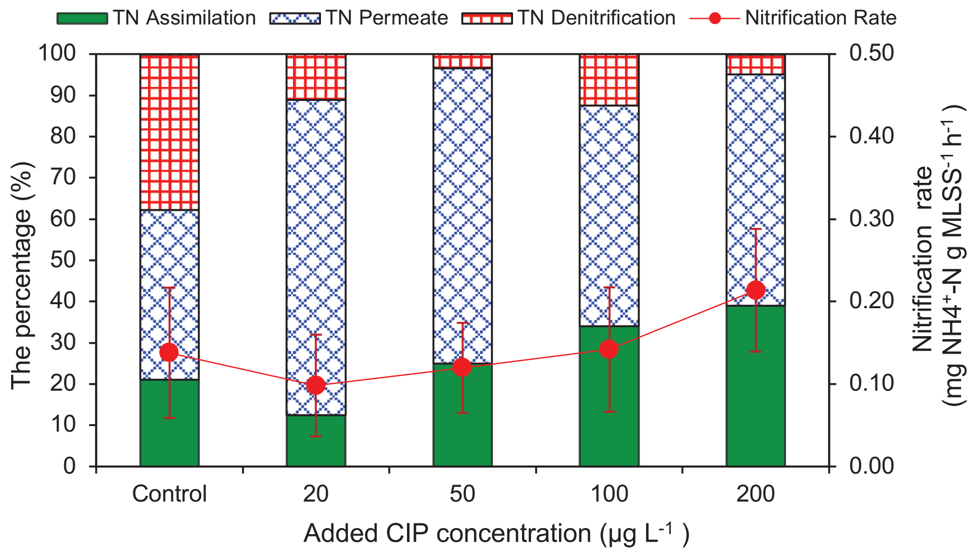

Figure 3. Effect of the CIP dosages on the nitrogen removal

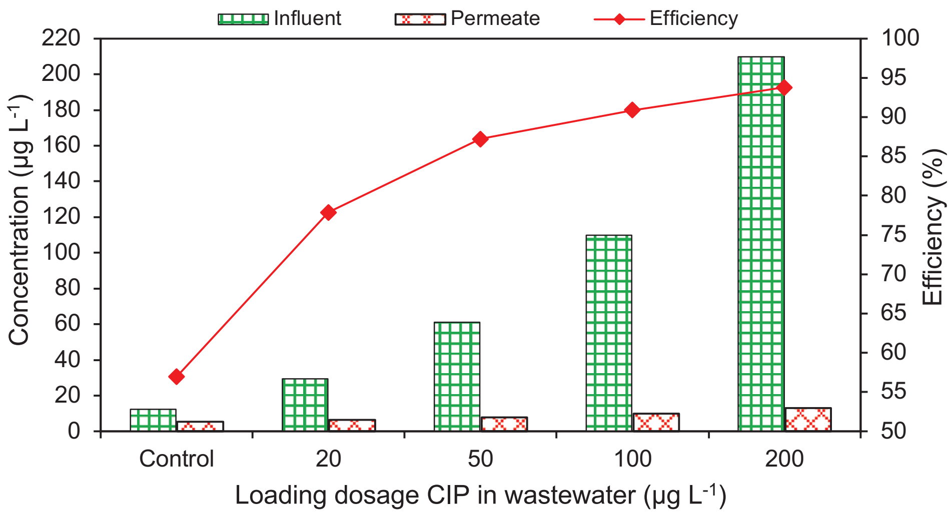

Figure 4. CIP removal at operating dosages

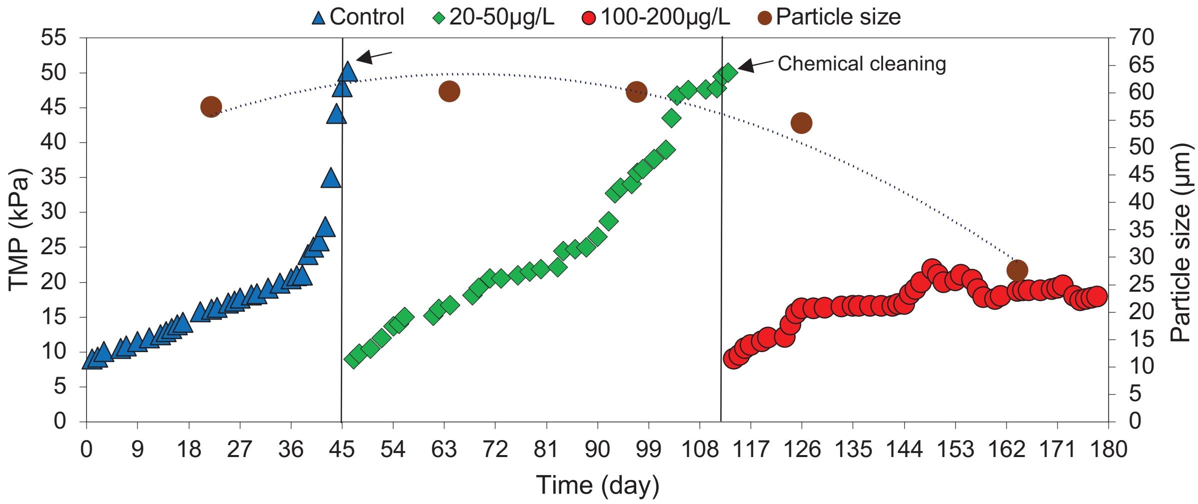

Figure 5. Effect of CIP dosages on fouling propensity and particle size in Sponge-MBR

Table 1. Performance of COD removal under various CIP dosages in Sponge-MBR

Parameters	CIP dosage				
	Control (No added CIP dosage)	20 μg L^{-1}	50 μg L^{-1}	100 μg L^{-1}	200 μg L^{-1}
Feed (mg L^{-1})	344 ± 64	350 ± 67	467 ± 93	417 ± 172	394 ± 92
Permeate (mg L^{-1})	12 ± 7	22 ± 9	11 ± 5	13 ± 8	22 ± 13
Efficiency (%)	96 ± 2	94 ± 2	98 ± 1	96 ± 3	94 ± 4
Removal rate (mg COD mg $MLSS^{-1}$ d^{-1})	0.19 ± 0.03	0.29 ± 0.06	0.31 ± 0.06	0.32 ± 0.13	0.26 ± 0.07

Table 2. Concentrations of nitrogen species in the feed

Parameters	CIP dosage				
	Control (No added CIP dosage)	20 µg L^{-1}	50 µg L^{-1}	100 µg L^{-1}	200 µg L^{-1}
TKN (mg L^{-1})	23.4 ± 4.1	22.6 ± 5.0	24.8 ± 6.7	21.8 ± 8.9	18.8 ± 4.0
NH_4^+-N (mg L^{-1})	7.3 ± 3.5	7.3 ± 2.7	7.4 ± 2.5	5.8 ± 1.7	9.4 ± 2.0
NO_3^--N mg L^{-1})	0.3 ± 0.2	0.2 ±0. 1	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.2
NO_2^--N (mg L^{-1})	1.4 ± 1.9	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.5 ± 0.3
TN (mg L^{-1})	25.1 ± 4.7	23.1 ± 5.1	25.4 ± 6.7	22.3 ± 9.0	19.6 ± 4.1

Table 3. Concentrations of nitrogen species in the permeate

Nitrogen species	CIP dosage				
	Control (No added CIP dosage)	20 µg L^{-1}	50 µg L^{-1}	100 µg L^{-1}	200 µg L^{-1}
TKN (mg L^{-1})	5.2 ± 2.0	9.2 ± 2.5	7.8 ± 4.4	6.3 ± 4.3	5.0 ± 1.8
NH_4^+-N (mg L^{-1})	1.6 ± 0.5	4.3 ± 2.0	3.3 ± 2.2	1.9 ± 0.9	2.3 ± 1.8
NO_3^--N (mg L^{-1})	4.7 ± 4.9	7.4 ± 3.6	9.5 ± 4.1	4.1 ±3.7	5.5 ± 3.3
NO_2^--N (mg L^{-1})	0.5 ± 0.5	1.0 ± 1.6	0.9 ± 1.8	0.5 ± 0.7	0.6 ± 0.9
TN (mg L^{-1})	10.4 ± 5.3	17.6 ± 5.2	18.2 ± 6.1	10.9 ± 4.1	11.1 ± 4.7

Table 4. Membrane resistances at various CIP dosages

	Parameters	CIP dosage		
		Control (No added CIP dosage)	20-50 μg L^{-1}	100-200 μg L^{-1}
Resistance (m^{-1})	R_t	7.85E+12	7.15E+12	3.08E+12
	R_c	5.01E+12	5.01E+12	1.13E+12
	R_f	2.51E+12	1.78E+12	1.52E+12
	R_m	3.33E+11	3.65E+11	4.28E+11
Percentage (%)	R_c	63.8	70.1	36.6
	R_f	31.9	24.8	49.5
	R_m	4.2	5.1	13.9