

Application of a moving-bed biofilm reactor for sulfur-oxidizing autotrophic denitrification

Yan-Xiang Cui, Di Wu, Hamish R. Mackey, Ho-Kwong Chui and Guang-Hao Chen

ABSTRACT

Sulfur-oxidizing autotrophic denitrification (SO-AD) was investigated in a laboratory-scale moving-bed biofilm reactor (MBBR) at a sewage temperature of 22 °C. A synthetic wastewater with nitrate, sulfide and thiosulfate was fed into the MBBR. After 20 days' acclimation, the reduced sulfur compounds were completely oxidized and nitrogen removal efficiency achieved up to 82%. The operation proceeded to examine the denitrification by decreasing hydraulic retention time (HRT) from 12 to 4 h in stages. At steady state, this laboratory-scale SO-AD MBBR achieved the nitrogen removal efficiency of 94% at the volumetric loading rate of $0.18 \text{ kg N} \cdot (\text{m}^3_{\text{reactor}} \cdot \text{d})^{-1}$. The biofilm formation was examined periodically: the attached volatile solids (AVS) gradually increased corresponding to the decrease of HRT and stabilized at about $1,300 \text{ mg AVS} \cdot \text{L}_{\text{reactor}}^{-1}$ at steady state. This study demonstrated that without adding external organic carbon, SO-AD can be successfully applied in moving-bed carriers. The application of SO-AD MBBR has shown the potential for sulfur-containing industrial wastewater treatment, brackish wastewater treatment and the upgrading of the activated sludge system. Moreover, the study provides direct design information for the full-scale MBBR application of the sulfur-cycle based SANI process.

Key words | biofilm technology, MBBR, sulfate reduction autotrophic denitrification nitrification integrated (SANI) process, sulfur-oxidizing autotrophic denitrification (SO-AD)

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INTRODUCTION

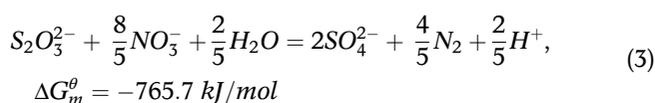
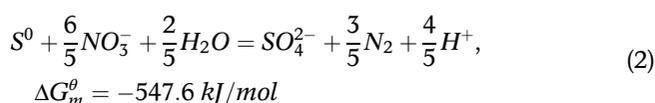
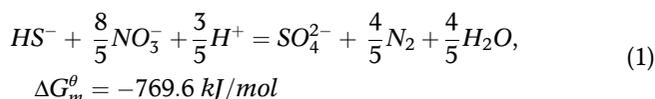
The moving-bed biofilm reactor (MBBR) is well known as a compact and robust biofilm technology. It has been extensively studied and used in the past three decades (Ødegaard 2016). This technology originated for organics removal in the late 1980s in Norway in cold climates where biomass growth is limited. Afterwards, it was developed to treat various types of wastewater, including domestic wastewater (Ødegaard 2016) and industrial wastewaters, such as the wastewater from dairy, pulp and paper and aquaculture industries (Rusten *et al.* 2006), and even wastewater containing toxic compounds and landfill leachate. Today, more than 1,200 full-scale MBBR plants operate around the world (Boltz *et al.* 2017).

Compared to the conventional activated sludge process, MBBR technology has the following special features: i) the functional bacteria grow on the protected surface of carriers, hence eliminating sludge recirculation, minimizing the

solids in the effluent of the bioreactor, and the size of secondary sedimentation tanks; and ii) the carriers are capable of freely moving in the reactor (like suspended sludge), consequently improving mass transfer and increasing the loading capacity, as well as reducing the bioreactor footprint (Ødegaard 2006; Rusten *et al.* 2006). More importantly, MBBRs use carriers with large protected specific surface areas (from 500 up to $3,000 \text{ m}^2 \cdot \text{m}^{-3}$), which is good for bacteria survival and biofilm formation (Ødegaard 2016). The protected surface provided by MBBR carrier is excellent for cultivating slow-growing bacteria (Bassin *et al.* 2012), such as nitrifiers (Hoang *et al.* 2014) and Anammox bacteria (Gilbert *et al.* 2014).

Notably, sulfur-oxidizing bacteria (SOB) are also slow growers with an observed yield of $0.15\text{--}0.57 \text{ g VSS} \cdot \text{g}^{-1} \text{ N}$ (Yang *et al.* 2016). SOB can utilize inorganic reduced sulfur compounds (e.g. S^{2-} , S^0 , $\text{S}_2\text{O}_3^{2-}$) as electron donors

to reduce nitrate, thereby eliminating the need for organic carbon oxidation (Cardoso *et al.* 2006). The stoichiometry of the denitrification reactions of nitrate is presented below for sulfide, elemental sulfur and thiosulfate as electron donors in Equations (1–3) (Cardoso *et al.* 2006).



Therefore, this process is called sulfur-oxidizing autotrophic denitrification (SO-AD). Because there is no need to add external organic carbon and less sludge is produced (Cardoso *et al.* 2006), the interest in applying SO-AD for wastewater denitrification has increased recently. For instance, simultaneous sulfide removal and denitrification was accomplished in treating oil-refining wastewater (Reyes-Avila *et al.* 2004; Vaiopoulou *et al.* 2005). Manconi *et al.* (2007) investigated the SO-AD in a bioaugmented activated sludge system, demonstrating that SO-AD was a suitable alternative to conventional heterotrophic denitrification for wastewater with low C/N ratio. SO-AD was also successfully applied for desulfurization and denitrification in simulated oil reservoir wastewater treatment (An *et al.*

2010). Furthermore, an immobilized sulfur-oxidizing culture was tested for SO-AD during anoxic biogas desulfurization (Fernández *et al.* 2014). However, these studies were investigated for either industrial wastewater treatment, or the enrichment of sulfur-oxidizing denitrifiers. Few applications were found in nitrogen removal of municipal wastewater treatment via SO-AD.

Our previous studies have developed SO-AD for saline sewage treatment (Lau *et al.* 2006). In Hong Kong, seawater has been applied for toilet flushing since the 1950s, saving about 22% of the city's fresh water demand (Lu *et al.* 2012a). Seawater contains a large amount of sulfate (on average 900 mg Sulfate-S·L⁻¹) (Wang *et al.* 2009). As a result, the saline sewage contains about 167 mg S·L⁻¹ of sulfate (Lu *et al.* 2012a). By making use of sulfate as electron carrier, a saline wastewater treatment process has been developed, the sulfate reduction autotrophic denitrification nitrification integrated (SANI) process (Wang *et al.* 2009). In SANI process (Figure 1), chemical oxygen demand (COD) can be degraded by sulfate-reducing bacteria under anaerobic conditions and sulfate is reduced to hydrogen sulfide, remaining dissolved in water. In a second reactor, the ammonia is oxidized to nitrate/nitrite under aerobic conditions. SO-AD is utilized as an intermediate reaction to loop the above two reactions into an integrated process (Figure 1) with minimal sludge production.

The potential of applying SANI process for saline sewage treatment has been demonstrated in a previous pilot test (Lu *et al.* 2012a) with a fixed bed anoxic reactor. However, the volumetric rate of this SO-AD was 0.1 kg N·(m³·d)⁻¹, which is only around half of the rate obtained in an SOB augmented activated sludge system (Manconi

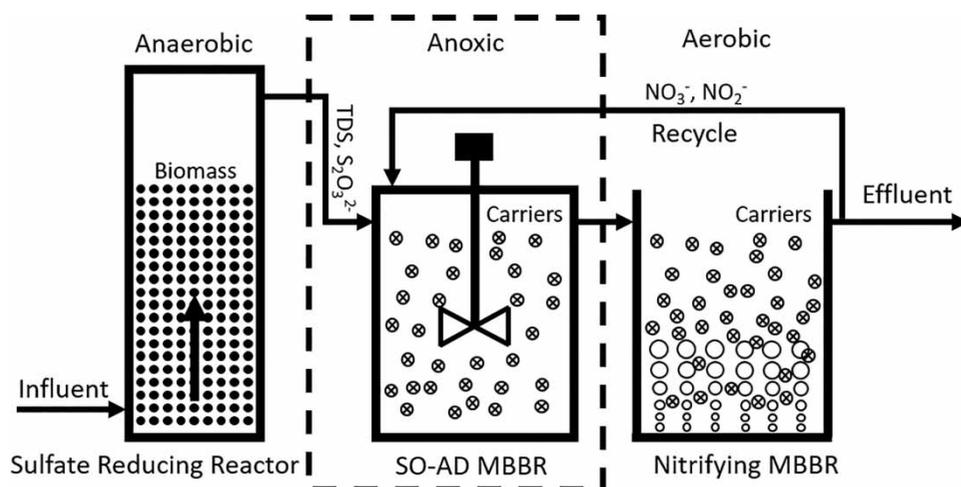


Figure 1 | Schematic flowchart of SANI process. The SO-AD MBBR is shown in the frame with dashed line.

et al. 2007). The lower rate of SO-AD in the SANI pilot plant was caused by a quite low sludge concentration ($\leq 300 \text{ mg VSS} \cdot \text{L}_{\text{reactor}}^{-1}$) sustained in its conventional fixed-bed anoxic reactor even after 200 days' operation (Lu *et al.* 2012a, 2012b). By considering the process scale-up for full-scale application, it is critical to develop a new reactor technology for SO-AD, which can retain an active and high concentration of SOB.

MBBR is a potential technology to enhance the SO-AD process due to its many benefits. However, key uncertainties exist before it can be applied, including whether SOB biofilm can be successfully developed on the surface of moving carriers, the removal efficiencies of sulfur and nitrogen compounds, and their associated removal rates. Thus, the present paper reports the bench-scale investigation of SO-AD biofilm formation and its performance in an MBBR system. The potential of implementing the SO-AD MBBR is also discussed.

METHODS

Laboratory-scale MBBR

A tightly sealed laboratory-scale MBBR was made of plexiglas with a height of 460 mm and a diameter of 180 mm (working volume of 10 L). A mechanical mixer provided good mixing at $80 \pm 10 \text{ RPM}$ (with the G value of 17 s^{-1}) in the reactor. The anoxic biological sulfide oxidation may result in the formation of elemental sulfur (Reyes-Avila *et al.* 2004; Cardoso *et al.* 2006), which could increase the probability of carrier clogging, especially in carriers with a specific surface area larger than $500 \text{ m}^2 \cdot \text{m}^{-3}$ (Forrest *et al.* 2016). Therefore, K₃ media was selected in the present experiment and the reactor was filled to 60% (Rusten *et al.* 2006). The K₃ media was made of high density polyethylene with a diameter of 25 mm, a height of 10 mm, and a specific protected surface area of $500 \text{ m}^2 \cdot \text{m}^{-3}$ (Rusten *et al.* 2006;

Gilbert *et al.* 2014; Hoang *et al.* 2014). The reactor was covered by light-tight cloth to eliminate the interference of phototrophic reaction.

Synthetic sewage

The synthetic saline sewage consisted of two parts, Solution A and Solution B, due to the instability of certain compounds that required regular preparation. The composition of the Solution A stock was modified from our previous research (Lau *et al.* 2006) with the main components of NH_4Cl ($18.37 \text{ g} \cdot \text{L}^{-1}$), K_2HPO_4 ($1.92 \text{ g} \cdot \text{L}^{-1}$), KH_2PO_4 ($0.72 \text{ g} \cdot \text{L}^{-1}$) and KNO_3 ($43.33 \text{ g} \cdot \text{L}^{-1}$) and trace metals as described in Lau *et al.* (2006). The stock was diluted 100 times to provide Solution A as used in the reactor. Solution B provided the sulfur and alkalinity and consisted of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ ($0.72 \text{ g} \cdot \text{L}^{-1}$), $\text{Na}_2\text{S}_2\text{O}_3$ ($0.12 \text{ g} \cdot \text{L}^{-1}$) and NaHCO_3 ($2 \text{ g} \cdot \text{L}^{-1}$). Given the instability of the sulfide and thiosulfate, Solution B was prepared daily and stored at 4°C . Both Solutions A and B were prepared with 80% deionized water and 20% seawater to simulate the same salinity of the saline wastewater (Wang *et al.* 2009). During the operation, Solutions A and B were equally mixed ($Q_A:Q_B = 1:1$) prior to being fed into the reactor. The sulfide, thiosulfate and nitrate concentrations of the synthetic sewage are shown in Table 1, in order to simulate the nitrogen removal of municipal wastewater treatment in a real application.

Reactor operation

This reactor was continuously operated in four stages over 240 days. In Stage I (Days 0–60), the reactor was started up with $20 \text{ L} \cdot \text{d}^{-1}$ of influent, corresponding to the reactor hydraulic retention time (HRT) equal to 12 h (Table 2). After a short transitional period of Stage II (Days 61–84) with the HRT of 8 h, the operation proceeded to further increase the nitrogen loading rate (NLR) (Table 2) in subsequent stages. In Stage III (Days 85–160) and Stage IV

Table 1 | Comparison of the concentrations of the major substrates in SO-AD between the current and previous studies of the SANI process

SO-AD reactor	TDS ($\text{mg S} \cdot \text{L}^{-1}$) ^a	Thiosulfate ($\text{mg S} \cdot \text{L}^{-1}$)	Nitrate ($\text{mg N} \cdot \text{L}^{-1}$)	$\text{COD}_{\text{sulfur}}/\text{N}$ (g COD/g N)	Reference
Laboratory-scale MBBR	45.3 ± 7.1	24.3 ± 5.7	29.8 ± 2.3	3.9 ± 0.7	Current study
Laboratory-scale biofilm reactor	36.6–70	120 ^b	30.3–30.9	2.44–4.62	Wang <i>et al.</i> (2009)
Large-scale MBBR	79 ± 5.8	–	7.9 ± 3.2	5 ^c	Wu <i>et al.</i> (2016)

^aTDS, total dissolved sulfide.

^bThe thiosulfate was fed to cultivate SOB during start-up phase.

^cBased on the recycle ratio ($Q_{\text{recycle}} \cdot Q_{\text{influent}} = 4$), $\text{COD}_{\text{sulfur}}/\text{N} = [\text{TDS}] \cdot Q_{\text{influent}} / [\text{NO}_3^-] \cdot Q_{\text{recycle}}$.

Table 2 | Operational conditions applied to SO-AD MBBR

Stage	HRT (h)	Nitrate (mg N·L ⁻¹)	TDS (mg S·L ⁻¹)	Thiosulfate (mg S·L ⁻¹)	NLR (kg N·(m ³ ·d) ⁻¹)	COD _{sulfur} /N (g COD/g N)
Stage I	12	31.0 ± 2.2	43.9 ± 10.9	22.7 ± 9.0	0.062 ± 0.004	3.7 ± 1.0
Stage II	8	29.2 ± 8.7	47.0 ± 6.5	26.3 ± 2.7	0.088 ± 0.026	4.3 ± 1.5
Stage III	4	28.8 ± 2.1	45.8 ± 2.4	25.0 ± 5.3	0.173 ± 0.013	4.0 ± 0.4
Stage IV	4	30.0 ± 2.1	45.7 ± 5.6	24.3 ± 3.9	0.180 ± 0.013	4.0 ± 0.4

(Days 161–240), the influent flow rate was increased further to 3 times the original rate in Stage I, resulting in the HRT of 4 h. Stages I–III described system start-up; and Stage IV presented the system performance under pseudo steady state condition. During the operation, the pH of the influent was maintained at 7.5 ± 0.3 by the addition of HCl (1M) or NaOH (1M); the dissolved oxygen (DO) kept below $0.1 \text{ mg O}_2\cdot\text{L}^{-1}$; and sewage temperature in the reactor was controlled at $22 \pm 1 \text{ }^\circ\text{C}$.

Analytical methods

Both influent and effluent samples were collected *in-situ* and filtered through $0.22 \mu\text{m}$ disposable Millipore filters prior to analysis of ions. Sulfate and thiosulfate were analyzed using ion chromatography (HIC-20A super, Shimadzu). Nitrate, nitrite, total dissolved sulfide (TDS), total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to the APHA standard methods (APHA 2005). The attached total solids (ATS, $\text{mg ATS}\cdot\text{L}^{-1}$) of the MBBR biofilm were measured according to Salvetti *et al.* (2006). The biomass were detached from the surface of carriers for determining the VSS/TSS ratio (APHA 2005), and hence the attached volatile solids (AVS, $\text{mg AVS}\cdot\text{L}^{-1}$) can be calculated. The surface morphology of biofilm was observed in fully hydrated condition by utilizing an inverted microscope (Olympus CX41) equipped with a digital camera (Olympus C550D Zoom).

RESULTS AND DISCUSSION

Biofilm formation

The challenge of the proposed MBBR technology is to develop SO-AD biofilm on moving carriers, mainly due to (i) the slow-growing nature of SOB bacteria (Yang *et al.* 2016) and (ii) the strong hydraulic shear in the MBBR tank, making accumulation of sufficient SOB on the carriers relative to solids detachment from the carrier surface difficult. Nevertheless, throughout the study SO-AD biofilm gradually formed in the laboratory-scale MBBR. Stereomicroscope and scanning electron microscope (the latter not shown), revealed an extremely thin (Figure 2(a)) biofilm at Day 30 with the external surface of the sample carrier essentially devoid of biomass; the biofilm then gradually grew thicker and the protected surface of the carrier (sample taken on Day 200) was fully occupied by the biomass, as shown in Figure 2(b).

Figure 3 shows the quantification of biomass growth attached on the MBBR carriers. Both the ATS and AVS continued increasing in the start-up and transitional periods (Stages I and II), and the AVS/ATS ratio of the SO-AD biofilm was maintained around 82% (Stage I) and 66% (Stage II), respectively. In Stage III, the AVS/ATS ratio fell in the range of 66–77%, and the AVS increased from $832 \pm 116 \text{ mg AVS}\cdot\text{L}^{-1}$ to $1,328 \pm 131 \text{ mg AVS}\cdot\text{L}^{-1}$, indicating the continuous enrichment of biomass. Finally, the AVS in

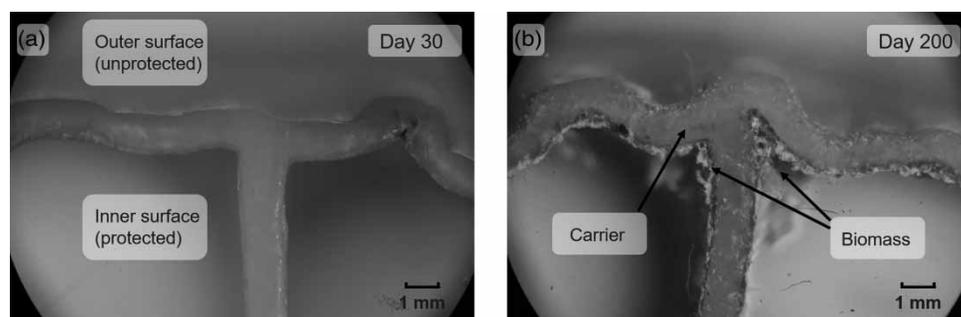


Figure 2 | Stereotype micrographs ($\times 40$ magnification) of carrier: on Day 30 (a) and Day 200 (b).

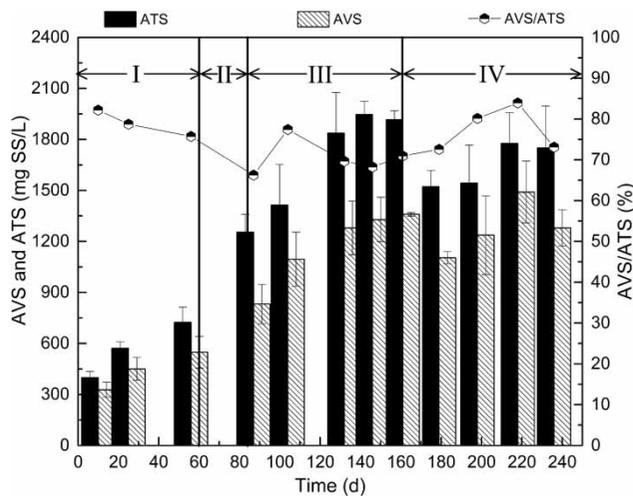


Figure 3 | Sludge concentration of attached total solids (ATS), attached volatile solids (AVS) and AVS/ATS ratio.

Stage IV stabilized at $1,294 \pm 144 \text{ mg AVS}\cdot\text{L}^{-1}$ with the ratio of AVS/ATS at $77 \pm 5\%$. This result indicates that the biofilm system of Stage IV achieved equilibrium between the growth and decay of microorganisms as well as solid attachment and detachment from the carriers (Liu & Tay 2002).

In our experiments, the concentration of the VSS in the bulk liquid of the reactor were $19.5 \pm 16.1 \text{ mg VSS}\cdot\text{L}^{-1}$ in Stage I, $21.6 \pm 5.4 \text{ mg VSS}\cdot\text{L}^{-1}$ in Stage II, $25.8 \pm 8.7 \text{ mg VSS}\cdot\text{L}^{-1}$ in Stage III, and $31.7 \pm 11.2 \text{ mg VSS}\cdot\text{L}^{-1}$ in Stage IV, respectively. The above-mentioned concentrations are equivalent to only 5%, 3%, 2%, and 2% of the AVS in the four stages. Because the synthetic sewage contains minimal solids, the above-mentioned suspended solids most likely come from the biofilm slough-off and biomass from free-growing bacteria (Piculell *et al.* 2014); and the low fraction of the suspended solids-to-attached solids imply the SO-AD reaction carried out by the bulk liquid biomass is negligible, as similar to the other autotrophic denitrification biofilm system (e.g. nitrifying or Anammox MBBR) (Piculell *et al.* 2014).

From the above, SO-AD biofilm was successfully developed under this current feeding regime (decreasing HRT), providing sufficient active biomass to carry out the SO-AD.

Reactor performance

The laboratory-scale MBBR reactor was continuously operated for more than 240 days, through start-up (Stage I), transitional periods (Stage II and Stage III) and stable operation (Stage IV). The performance of the reactor with

respect to the NO_x (NO_2^- and NO_3^-) reduction and the reduced sulfur (sulfide and thiosulfate) oxidation is illustrated in Figures 4 and 5.

Nitrogen reduction

The operation was run at the HRT of 12 h in Stage I and the relatively high HRT provided the reactor with a corresponding low wash-out rate with the purpose of cultivating the functional microorganisms on the surface of the K_3 carrier. This start-up measure is commonly used for slow-growing biofilm system (Bassin *et al.* 2012). After a short period of start-up (approximately 20 days), the nitrogen removal rate gradually increased to an average value of $0.051 \text{ kg N}\cdot(\text{m}^3\cdot\text{d})^{-1}$ and the nitrogen removal efficiency increased to around 82% from Day 21 (Figure 4(b)), indicating that the start-up period of denitrification in an SO-AD MBBR

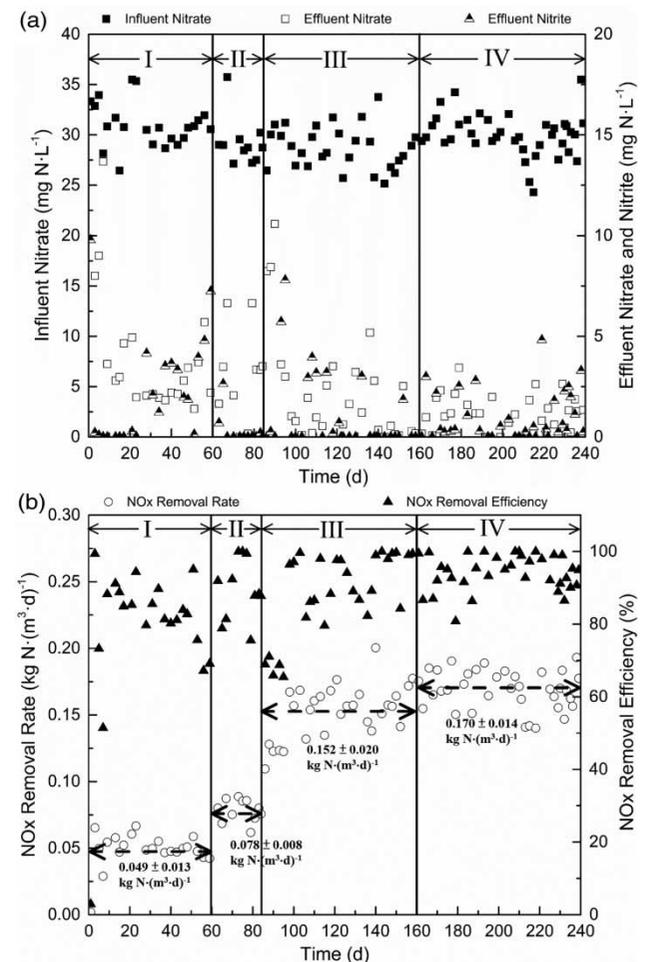


Figure 4 | The operational performance of the SO-AD MBBR on nitrogen reduction: influent and effluent nitrogen profiles (a); nitrogen removal rate and removal efficiency (b).

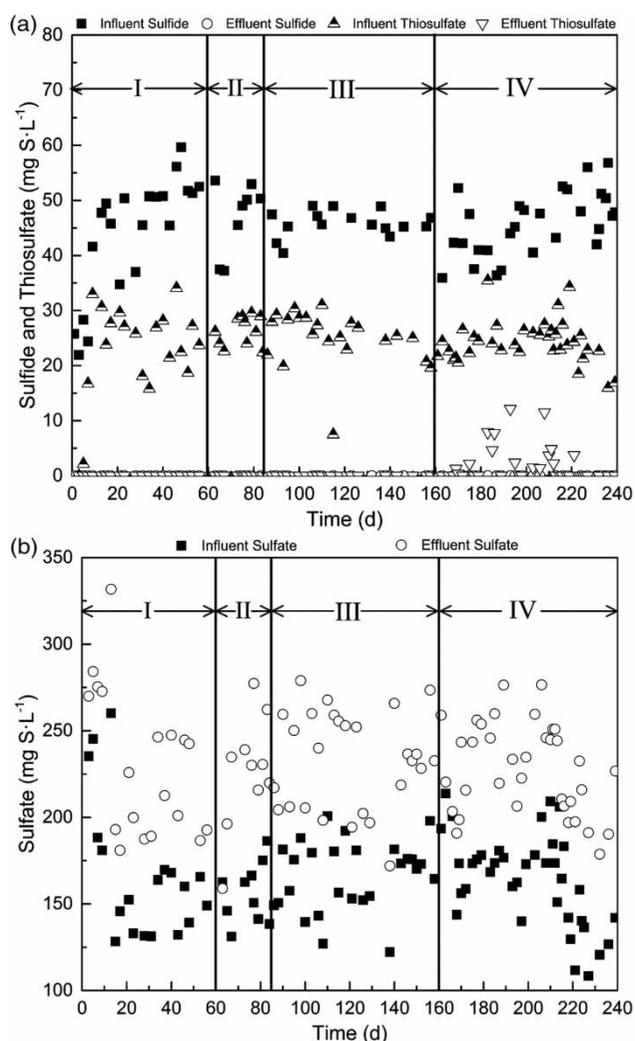


Figure 5 | The operational performance of the SO-AD MBBR on sulfur oxidation: Sulfide and thiosulfate profiles (a); Sulfate profile (b).

is relatively short. Although the biofilm was rather thin (i.e. low biomass concentration) (Figure 3) in Stage I, nitrogen removal was carried out satisfactorily, and the overall nitrogen removal rate averaged $0.049 \pm 0.013 \text{ kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$ (Figure 4(b)). The relatively fast development of the SO-AD biofilm is promising as, by comparison to the literature, the start-up of SO-AD suspended sludge systems is around 70 days (Manconi *et al.* 2006).

Following development of the thin biofilm and nitrogen removal capacity, the reactor underwent a decreasing HRT operation with corresponding increase in NLR. The NLR in Stage II was increased nearly 1.5 times that of Stage I (Table 2). During this transitional period, the nitrogen removal efficiency achieved $89.4 \pm 8.4\%$ and the nitrogen removal rate was maintained at around $0.078 \pm 0.008 \text{ kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$. Following this, the NLR was further increased

to $0.173 \pm 0.013 \text{ kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$ in Stage III. The effluent nitrate and nitrite were significantly increased (up to $6.77 \pm 3.31 \text{ mg N} \cdot \text{L}^{-1}$ and $2.76 \pm 3.73 \text{ mg N} \cdot \text{L}^{-1}$, respectively, Figure 4(a)) at the initiation of Stage III, resulting in the decrease of denitrification efficiency to 65–71%. Meantime, the nitrogen removal rate only increased to $0.120 \pm 0.007 \text{ kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$. However, within 10 days' acclimation (Days 85–95), the NO_x removal efficiency was quickly recovered to 82% or even higher with the nitrogen removal rate increased to $0.159 \pm 0.016 \text{ kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$. In Stage IV, the NO_x removal efficiency achieved $94 \pm 5\%$, which was significantly higher than that of the average value in Stage III ($89 \pm 11\%$). The effluent NO_x ($\text{NO}_3^- + \text{NO}_2^-$) was less than $2.0 \text{ mg N} \cdot \text{L}^{-1}$, which provided much better effluent quality than the previous stages (Figure 4(a)). Meanwhile, the highest nitrogen removal rate ($0.170 \pm 0.014 \text{ kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$) was shown in this period. All these results indicate the steady state of the operation in Stage IV.

Sulfur oxidation

The sulfide was entirely oxidized via nitrate/nitrite reduction in all four stages (Figure 5). Another reduced sulfur compound – thiosulfate – was completely consumed in Stages I, II and III, whereas in Stage IV a small amount of thiosulfate ($1.38\text{--}7.74 \text{ mg S} \cdot \text{L}^{-1}$) was detected in the effluent which may imply that sulfide was prioritized in the utilization of electron donors. That some thiosulfate remains indicates that there is an excess of electron donor. However, there was also a greater utilization of electron donor in Stage IV corresponding to a greater NO_x removal efficiency.

Theoretically, given the adequate amount of nitrate, all the reduced sulfur removed should be oxidized to sulfate (Equations (1–3)) as the final oxidized product; so the value of the increased sulfate (ΔSO_4) shall be identical to the sum of the oxidized sulfide and thiosulfate. Our results showed that in Stage I, the increased sulfate concentration ($61.2 \pm 21.0 \text{ mg S} \cdot \text{L}^{-1}$) was lower than that of the reduced sulfur oxidized ($67.3 \pm 17.8 \text{ mg S} \cdot \text{L}^{-1}$). This is probably due to sulfide being oxidized to other forms of sulfur compounds (mostly elemental sulfur) (Reyes-Avila *et al.* 2004). The elemental sulfur can then be either washed away through effluent or absorbed by the biofilm. However, in the subsequent stages, the differences between the amount of sulfur oxidation and sulfate generation were gradually decreased. For instance, the values of the increased sulfate and the reduced sulfur oxidized were 71.3 ± 15.5 and $68.8 \pm 5.8 \text{ mg S} \cdot \text{L}^{-1}$. The result coincides with the increase of the sulfur balance percentage from $94.2 \pm 47.0\%$ in

Stage I to $105.8 \pm 28.1\%$ and $101.1 \pm 33.5\%$ in Stage III and IV, respectively.

It should be noted that the average sulfur-COD/ ΔNO_x of the present study is in the range of 4.65 ± 0.94 in Stage I to 4.07 ± 0.52 in Stage IV. This ratio is consistent with the COD/N ratio for biological denitrification. The electron donors utilized in the four stages are generally higher than the stoichiometric value as a result of sulfur-COD consumption for CO_2 fixation and the generation of cell intermediates (Manconi *et al.* 2006).

Implementation

In this study, the laboratory-scale MBBR achieved the NO_x removal efficiency of 94% under the NLR of $0.18 \text{ kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$ at the steady state. This NLR value was slightly higher than the number reported in an activated sludge system (Manconi *et al.* 2006) and comparable to the numbers for continuous flow stirred tanks (Reyes-Avila *et al.* 2004; Vaiopoulou *et al.* 2005) (Table 3). SO-AD MBBR has shown great potential for further application in industrial wastewater treatment and the upgrading of the activated sludge system.

In the SANI process treating brackish wastewater, this number almost doubles that of the earlier pilot plant (Lu *et al.* 2012b) and is close to the rate observed in a

laboratory-scale fixed-bed biofilm reactor (Wang *et al.* 2009). Note that after this laboratory-scale investigation, the MBBR-based SO-AD has moved forward to a large-scale SANI[®] demonstration plant at Shatin, Hong Kong, treating $800\text{--}1,000 \text{ m}^3$ of sewage per day (the details have been introduced in Wu *et al.* (2016)). The design parameters and start-up of the large-scale SO-AD MBBR tank are based on this laboratory investigation (Table 4).

CONCLUSIONS

This investigation demonstrated the feasibility of developing an MBBR for SO-AD in municipal wastewater treatment. During the stable state, denitrification was achieved along with the complete oxidation of reduced sulfur into sulfate. The surface specific denitrification and sulfur oxidation rates increased together with the increase of the biomass (AVS), coinciding with the occupation of the biomass on the protected surface of the carriers. The successful operation of the laboratory-scale SO-AD MBBR provided significant reference on the associated large-scale application of SANI process and the treatment of other sulfide-containing wastewaters.

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Table 3 | The comparison of NLR (SO-AD) in the literature

Reactors of SO-AD	NLR ($\text{kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$)	Reference
MBBR	0.06–0.18	Current study
Activated sludge reactor	0.007–0.138	Manconi <i>et al.</i> (2006)
CSTR	0.127–0.282	Vaiopoulou <i>et al.</i> (2005)
CSTR	0.2	Reyes-Avila <i>et al.</i> (2004)
Upflow biofilm reactor	~0.1	Lu <i>et al.</i> (2012b)
Upflow fixed-bed reactor	0.18–0.3	Wang <i>et al.</i> (2009)

Table 4 | The design criteria obtained from the laboratory-scale SO-AD MBBR

Reactor of SOAD	HRT (h)	NLR ($\text{kg N} \cdot (\text{m}^3 \cdot \text{d})^{-1}$)	Filling fraction (%) ^a
MBBR	4	0.18	60

^aFilling fraction = $V_{\text{carrier}}/V_{\text{reactor}} \times 100\%$ (Rusten *et al.* 2006; Gilbert *et al.* 2014).

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