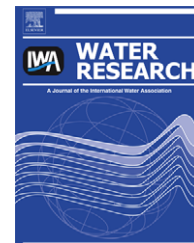


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A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment

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ABSTRACT

This paper reports on a lab-scale evaluation of a novel and integrated biological nitrogen removal process: the sulfate reduction, autotrophic denitrification and nitrification integrated (SANI) process that was recently proposed for saline sewage treatment. The process consisted of an up-flow anaerobic sludge bed (UASB) for sulfate reduction, an anoxic filter for autotrophic denitrification and an aerobic filter for nitrification. The experiments were conducted to evaluate the performance of the lab-scale SANI system with synthetic saline wastewater at various hydraulic retention times, nitrate concentrations, dissolved oxygen levels and recirculation ratios for over 500 days. The system successfully demonstrated 95% chemical oxygen demand (COD) and 74% nitrogen removal efficiency without excess sludge withdrawal throughout the 500 days of operation. The organic removal efficiency was dependent on the hydraulic retention time, up-flow velocity, and mixing conditions in the UASB. Maintaining a sufficient mixing condition in the UASB is important for achieving effective sulfate reduction. For a typical Hong Kong wastewater composition 80% of COD can be removed through sulfate reduction. A minimum sulfide sulfur to nitrate nitrogen ratio of 1.6 in the influent of the anoxic filter is necessary for achieving over 90% nitrate removal through autotrophic denitrifiers which forms the major contribution to the total nitrogen removal in the SANI system. Sulfur balance analyses confirmed that accumulation of elementary sulfur and loss of hydrogen sulfide in the system were negligible.

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1. Introduction

In Hong Kong the capacity of the landfills for sewage sludge will be suppressed in 2015. Sludge incineration seems to be a last remaining option for Hong Kong. However, it would impact the rapidly deteriorating air quality as well as create a problem of locating the incinerator without large public discontent. Reduction in the volume of sewage sludge is a first

step in the solution of the sludge problem. Anaerobic digestion of secondary sludge hardly decreases the sludge and construction of more sludge digesters is a problem in Hong Kong because of limited available land. In this respect, reduction of sludge within the treatment works without the need for extra space is ideal for Hong Kong.

Various sludge-minimizing options have been studied, such as disintegration of excess sludge by thermal, ultrasonic

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and ozone pretreatments (Nickel et al., 1998; Rocher et al., 2001; Saby et al., 2002) or modification of a biological nitrogen removal (BNR) process into an oxic-settling-anaerobic (OSA) process by inserting a sludge holding tank in the sludge return line between its final clarifier and bioreactor (Saby et al., 2002, 2003; An and Chen, 2008). However, these options lead to either high costs or the need for more space. The best option for sludge minimization is using low sludge production processes. For chemical oxygen demand (COD) conversion of low sludge production can be achieved by using low quality electron acceptors instead of oxygen or nitrate. Combination of denitrification and methanogenesis has been proposed (Akunna et al., 1994; Hendriksen and Ahring, 1996; Del Pozo and Diez, 2003). In such systems, heterotrophic denitrification and methanogenesis both occur in the anaerobic reactor. As the sludge production from heterotrophic denitrification (0.4 g volatile suspended solids (VSS)/g COD) is much higher than that from anaerobic reactions (0.1 g VSS/g COD), diversion of the substrate from methanogenesis towards denitrification increases the overall sludge production (Inamori et al., 1996). In addition, methanogenesis is much slower than denitrification, which requires a very long hydraulic retention time (HRT) to remove COD efficiently.

Besides methanogenesis, sulfate reduction also leads to a low sludge yield (Lens et al., 1995, 1998) because the growth yield of sulfate-reducing bacteria (SRB) is only 0.2 g VSS/g reduced sulfate (Kleerbezem and Mendez, 2002; van den Bosch et al., 2007). The minimal COD requirement in sulfate reduction is 2 g of COD consumed per g of $\text{SO}_4^{2-}\text{-S}$ reduced. Hong Kong sewage contains 500 mg/L sulfate or 167 mg/L $\text{SO}_4^{2-}\text{-S}$ and 400 mg/L COD, indicating a sufficient reduction potential. SRB can out-compete methane producing bacteria (MPB) for organic substrates because of their higher specific growth rate and lower Monod's saturation coefficient than that of MPB (Widdel, 1988). In a closed anaerobic environment, hydrogen sulfide generated from sulfate reduction tends to dissolve in water as pH increases (Harada et al., 1994), thereby generating adequate amounts of dissolved sulfide. It is known that various sulfur sources are able to serve as electron donors for autotrophic denitrification to remove nitrate (Kleerbezem and Mendez, 2002; van den Bosch et al., 2007). Since the growth

yield of autotrophic denitrifiers is low, combining sulfate reduction and denitrification based on sulfide leads to low net sludge production.

Based on the above rationale, we have recently developed the sulfate reduction, autotrophic denitrification and nitrification integrated (SANI) process for low-cost reduction of excess sludge (Lau et al., 2006; Tsang et al., in press). This new BNR process significantly reduces excess sludge production because the three major microbial populations in the process: SRB, autotrophic denitrifiers and nitrifiers all have low growth yields. In this process, most of the COD is oxidized to CO_2 in sulfate reduction by SRB. By considering the full process design, we estimate that the total cost reduction would be more than 50% for a 10,000 m^3 /day sewage treatment works (unpublished data). Fig. 1 shows a schematic diagram of the experimental setup of the SANI system. It consists of (1) an anaerobic zone to remove COD by SRB; (2) a subsequent anoxic zone for autotrophic denitrification of nitrate with dissolved sulfide generated from sulfate reduction integrated with (3) an aerobic zone to nitrify ammonia and recirculate nitrate to the anoxic zone for the denitrification. It should be noted that phosphorus removal is not mandatory in Hong Kong since all sewage treatment effluents are discharged to the sea. The major challenges we face in the SANI process are the (1) effectiveness of sulfate reduction, (2) effectiveness of the autotrophic denitrification using dissolved sulfide; (3) performance of the process with respect to COD and total nitrogen (TN) removal and excess sludge production; (4) impact of the recirculation flow on autotrophic denitrification; and (5) accumulation of sulfur in the system. A comprehensive study was conducted to deal with these challenges. It was divided into three parts: (1) a lab-scale demonstration of the process with synthetic saline wastewater; (2) development of a steady-state model for process evaluation and (3) a pilot-trial of the process with real saline sewage. Parts (1) and (2) were completed in the last five years, while Part (3) is currently conducted. This paper reports on the laboratory study. The objective of this study was to develop a lab-scale setup of the SANI process to examine the effectiveness of sulfate reduction and autotrophic denitrification and to test the performance of the entire

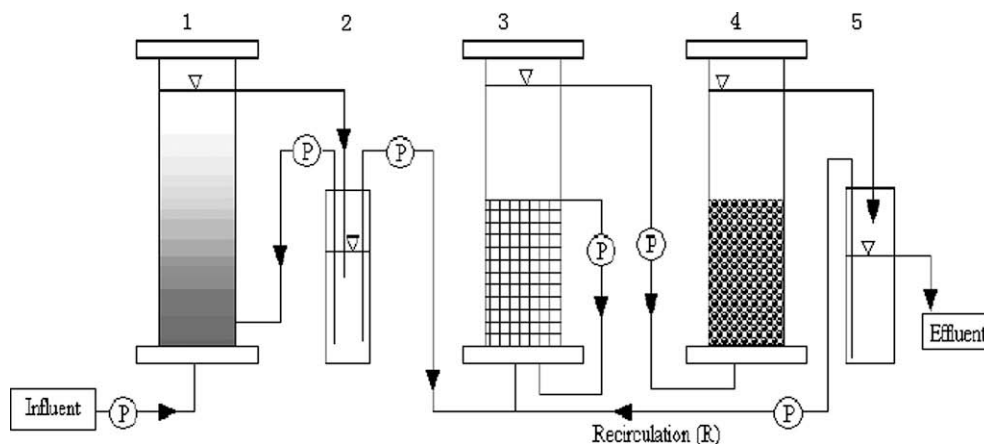


Fig. 1 – A schematic diagram of the experimental setup of the SANI system. (1) Up-flow anaerobic sludge blanket, (2) flow adjusting tank, (3) anoxic filter, (4) aerobic filter, and (5) effluent tank.

integrated process in terms of COD and nitrogen removal and sludge reduction.

2. Materials and methods

2.1. Experimental setup

Fig. 1 shows the lab-scale SANI system. An up-flow anaerobic sludge bed (UASB) was used as the sulfate reduction reactor. It was made with 100 mm in diameter and 400 mm in height and covered on both ends with plastic plates held together by stainless steel fasteners. Rubber O-rings were used to seal the contacts between the plates and the cylinder to make the cylinder complete airtight. The reactor had a liquid volume of 33.2 L plus a headspace of 0.5 L. An anoxic filter had a diameter of 100 mm and a height of 285 mm and was packed with polypropylene plastic media (specific surface area: 215 m²/m³). The reactor had a liquid capacity of 2.2 L and a headspace of 0.5 L. An aerobic filter was identical to the anoxic filter but with an air diffuser at the bottom.

2.2. Synthetic saline wastewater

A stock solution was prepared following our previous study (Lau et al., 2006) with the main components of glucose (19.57 g/L), sodium acetate (26.1 g/L), yeast extract (9.786 g/L), NH₄Cl (18.37 g/L), and K₂HPO₄ (1.92 g/L), KH₂PO₄ (0.72 g/L) and other trace metals. To simulate the characteristics of Hong Kong's sewage in terms of salinity and sulfate concentration, seawater (an average sulfate concentration of 2700 mg/L) was mixed with the stock solution and tap water proportionally (1:4.4 in volume) to achieve desired influent concentrations of COD, sulfate and nitrogen (265 mg COD/L, 500 mg SO₄²⁻/L (167 mg S/L) and 30 mg N/L ammonium nitrogen on average).

2.3. Sample analysis

During the experiments at each stage, the influent and effluent of all reactors were collected regularly. Because of the high salinity and sulfate in the feed, chloride and sulfide ions interfered with the COD measurements. TOC was analyzed instead of COD to determine the organic strength of the samples. TOC analysis was conducted with a Total Organic Carbon Analyzer (Shimadzu TOC-5000A) equipped with an auto-sampler. Potassium phthalate and sodium carbonate/sodium bicarbonate were used as the organic and inorganic calibration standards, respectively. In order to establish a correlation between TOC and COD, measurements of COD in a diluted synthetic stock solution were routinely conducted. We found an average ratio of 2.65 g COD–1 g TOC, from which COD values were determined. Nitrite, nitrate and sulfate were analyzed by an ion chromatograph (DIONEX-100) equipped with a conductivity detector and an IonPac AS9-HC analytical column. Dissolved sulfide was measured using an iodometric method (APHA, 2005). Suspended solids (SSs) and pH were determined according to the *Standard Methods* (APHA, 2005). Alkalinity was measured by titration (Neytzell-DeWilde et al., 1977; Moosbrugger et al., 1992) and dissolved oxygen (DO) was measured with a DO meter (YSI).

2.4. Reactor inoculation and system operation

2.4.1. Inoculations

All three reactors were inoculated with activated sludge obtained from a local sewage treatment plant and cultivated separately in a temperature-controlled chamber at 30 °C.

2.4.2. Stage I: separate operation of UASB and anoxic filter

The UASB reactor was initially subjected to a continuous feeding of the synthetic saline wastewater at a nominal hydraulic retention time (HRT) of 8 h. Internal recirculation was used to maintain well mixing conditions at the bottom of the sludge bed. In the same period, the anoxic filter was inoculated by feeding sodium nitrate and sodium thiosulfate with corresponding concentrations of 30 mg N/L and 120 mg S/L, respectively, along with other essential nutrients and trace minerals. An internal recirculation ratio of 2 was maintained for an effective substrate transfer between the bulk liquid phase and the biomass in the anoxic filter.

2.4.3. Stage II: integrative operation of UASB and anoxic filter

When the effluents of UASB and anoxic filter became stable, the anoxic filter was connected to the UASB and received its effluent with the addition of 30 mg NO₃-N/L. At this stage the effect of HRT on the COD removal, nitrate removal, sulfate reduction and sulfide oxidation by autotrophic denitrification was studied when the integration became stable. The operation conditions and performance of both UASB and anoxic filter in these runs are shown in Tables 1 and 2, respectively. Effect of influent nitrate concentration on the performance of the anoxic filter was also investigated.

2.4.4. Stage III: operation of aerobic filter

After more than 360 days of operating the UASB and anoxic filter together, the aerobic filter was fed with a continuous feeding of synthetic saline wastewater with an initial HRT of 12 h, which was gradually reduced to 2 h to increase the nitrogen loading. The aerobic filter biomass cultivation lasted for 80 days before the full nitrification capability was obtained and the filter was then connected to the UASB and anoxic filter integration to establish a complete SANI system.

Table 1 – The operating conditions of each reactor of the UASB/anoxic filter integrated setup under different HRTs.

Reactor	Run	I	II	III	IV
UASB	HRT (h)	6	4	3	3
	Internal recycling ratio (IR)	4	5	5	7
	Organic loading rate (kg TOC/m ³ /d)	0.42	0.64	0.86	0.86
	Up-flow velocity (m/h)	0.33	0.61	0.82	1.09
Anoxic filter	HRT (h)	/	4	2	2
	IR	/	2	2	2
	Nitrate loading rate (kg N/m ³ /d)	/	0.18	0.36	0.36
	Up-flow velocity (m/h)	/	0.21	0.43	0.43

Table 2 – The characteristics of the influents and effluents of each reactor of the UASB/anoxic filter integrated setup under different HRTs.

Reactor	Run	I	II	III	IV
UASB	Influent COD (mg/L)	248 ± 9	273 ± 13	270 ± 10	260 ± 8
	Effluent COD (mg/L)	44.3 ± 2.2	30.7 ± 1.2	54.9 ± 2.7	60 ± 2.4
	Influent sulfate (mg/L)	159 ± 8	185 ± 8	183 ± 9	185 ± 7
	Effluent sulfate (mg/L)	68 ± 3	78 ± 4	116 ± 2	97 ± 3
	Effluent dissolved sulfide (mg S/L)	60.7 ± 3	88.0 ± 4.1	49.2 ± 2.4	73.1 ± 3.1
	COD removal (%)	82.2 ± 4.1	88.7 ± 4.2	79.7 ± 3.9	77.0 ± 3.8
Anoxic filter	Influent nitrate (mg N/L)	/	30.3 ± 1.2	30.7 ± 1.5	30.9 ± 1.5
	Effluent nitrate (mg N/L)	/	0.78 ± 0.04	3.75 ± 0.12	0.36 ± 0.02
	Influent sulfate (mg/L)	/	60 ± 3	101 ± 5	87 ± 4
	Effluent sulfate (mg/L)	/	126 ± 6	134 ± 6	136 ± 6
	Influent dissolved sulfide (mg S/L)	/	70.0 ± 3.5	36.6 ± 1.8	66.4 ± 3.0
	Effluent dissolved sulfide (mg S/L)	/	5.0 ± 0.25	1.2 ± 0.06	13.2 ± 0.56
	Influent COD (mg/L)	/	24 ± 1.2	41 ± 2.0	51 ± 2.5
	Effluent COD (mg/L)	/	12 ± 0.60	10 ± 0.51	27 ± 1.2
	Nitrate removal (%) ^a	/	97.4 ± 4.8	87.8 ± 4.2	98.8 ± 4.3

a Nitrate removal(%) = NO₃ removed(g N)/Influent NO₃(g N) × 100.

2.4.5. Stage IV: operation of then SANI system

The aerobic filter was finally integrated into the UASB and anoxic filter integration to form the SANI system (see Fig. 1). The complete SANI setup was continuously fed with the synthetic saline wastewater. Mixing in all three reactors was achieved through respective internal recirculation (see Fig. 1). To provide a stable source of dissolved sulfide for the anoxic filter, the UASB was operated with a fixed HRT of 6 h during the start-up of the SANI system. The overall performance of the SANI system in terms of organic and nitrogen removal and excess sludge production was investigated at different ratios of the recirculation ratios between the anoxic and aerobic filter for improving the total nitrogen removal of the system.

3. Results

3.1. Performance of UASB

The main performance results of the UASB reactor in these four stages are reported in Fig. 2. The UASB was operated for more than 600 days with average organic and sulfate influent concentrations of 265 mg COD/L and 166 mg SO₄²⁻-S/L. In Stage 1, the UASB was operated at an HRT of 8 h. After a 60-day start-up period, the UASB achieved 80% COD removal. The up-flow velocity in the reactor was 0.2–0.3 m/h and formation of sulfidogenic granules was observed in the bed. In this anaerobic reactor, 45% of the sulfate was reduced to sulfide, of which 85% was present in dissolved form, producing an average dissolved sulfide effluent of 76.5 mg S/L.

After reaching a steady state, the HRT was reduced to 4 h and the anoxic filter was then connected to the UASB (Stage 2). The organic loading rate of the UASB therefore increased up to 1.7 kg COD/m³-d and the COD removal increased to 88.7%. This was due to enhancement in the sulfate reduction, which was confirmed by an increase in the sulfide concentration of the UASB effluent. The reduced HRT also resulted in a high up-flow velocity of 0.61 m/h, thereby increasing the sulfate reduction efficiency.

When the HRT of the UASB was further reduced to 3 h, the organic loading rate increased to 2.28 kg COD/m³-d and the organic removal efficiency of the UASB dropped to 79.7%. The dissolved sulfide concentration in the effluent was reduced to 9.2 mg/L with a corresponding sulfate removal of only 36%. The corresponding HRT in the anoxic filter decreased to 2 h and the nitrate removal declined significantly. With the intention of improving the process performance and increasing the dissolved sulfide production, the internal recirculation ratio was increased to 7, resulting in an up-flow velocity of 1.09 m/h in the bed. While the COD removal efficiency slightly improved, the sulfate reduction rate improved considerably as the dissolved sulfide concentration in the UASB effluent recovered to 73.1 mg S/L. This was probably due to the improvement of the substrate transfer between the bulk and attached biomass phases under a high up-flow velocity. This recovery also provided sufficient sulfides to the anoxic filter for maintaining the nitrate removal at 99%.

3.2. Performance of anoxic filter

Performance results of the anoxic filter in Stages 1, 2 and 4 are shown in Fig. 2. The anoxic filter performed well in the cultivation period as nitrate removal reached more than 95% at a nitrate loading rate of 0.31 kg NO₃-N/m³-d. In the UASB and anoxic filter integration, nitrate removal was mainly achieved through the autotrophic denitrification in the anoxic filter.

In the anoxic filter reactor, high nitrate removal efficiency was achieved, resulting in nitrate in the effluent of the anoxic filter below 1 mg/L even when the HRT was 3 h. No accumulation of nitrite was observed in the filter effluent, suggesting that nitrate was completely converted to nitrogen gas. Ninety-three percent of dissolved sulfide was oxidized to sulfate in this reactor at this HRT, with a 65.7-mg S/L sulfate recovery. These results clearly demonstrated that the dissolved sulfide in the UASB effluent could be effectively utilized in the autotrophic denitrification. Furthermore, only 13.1 mg/L COD on average was removed in the anoxic filter, confirming that denitrification

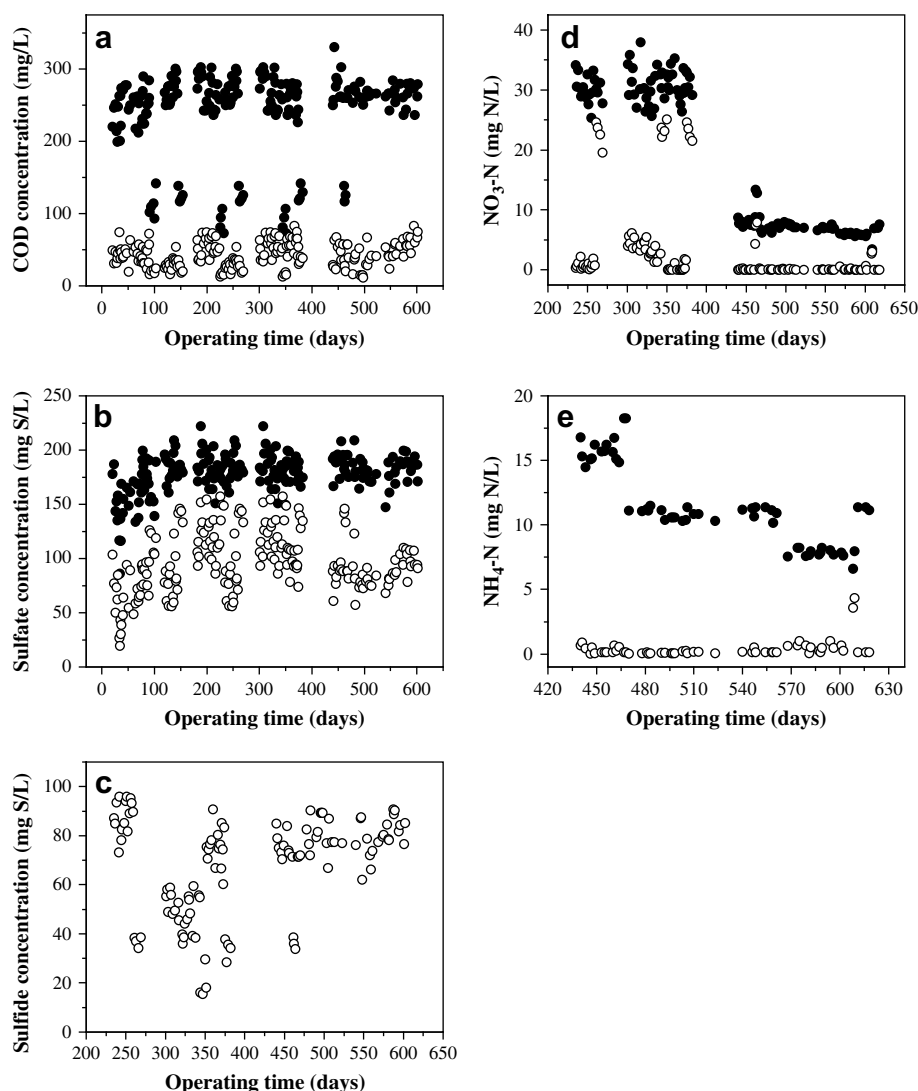


Fig. 2 – The performance of (a) COD removal, (b) sulfate reduction and (c) sulfide generation in the UASB, (d) nitrate removal in the anoxic filter, and (e) ammonia removal in the aerobic filter of the SANI system, respectively. The solid and open symbols signify the influent and effluent of each reactor, respectively.

through heterotrophic pathways was not significant. It should be noted that the dissolved sulfide concentration in the anoxic filter influent was around 10% lower than that in the UASB effluent due to the injection of the nitrate solution into the anoxic filter influent to provide a nitrate source for the UASB and anoxic filter integration (Stage 2).

The effect of the influent nitrate concentration on the anoxic filter is shown in Fig. 3. When the influent nitrate of the anoxic filter was below 30 mg N/L, the mean effluent nitrate was as low as 0.5 mg N/L; however, when it increased to 50 and 70 mg N/L, the corresponding effluent nitrate increased up to 10.3 and 28.5 mg/L, resulting in a decrease of the nitrate removal efficiency to 79 and 60%, respectively. In response to the increased influent nitrate concentrations at 50 and 70 mg N/L, 96 and 134 mg S/L, respectively, sulfides were stoichiometrically required for a complete autotrophic denitrification. However, the actual dissolved sulfide available in the influent was only 55–67 mg S/L. Clearly extra COD (sulfide) would be needed for a full denitrification. This could be

obtained by adding a waste sulfuric acid waste to the influent because there was still room for more sulfate reduction on the available COD in the influent.

3.3. Performance of aerobic filter

Performance of the aerobic filter is shown in Fig. 1. After 80 days inoculation, a full nitrification was achieved in this filter (data not shown). After being integrated into the SANI system, the filter performed well, maintaining the ammonia concentration in its effluent at around 1 mg N/L. The variation of the influent ammonia concentration was due to the dilution of the UASB effluent by the recirculation flow between the anoxic and aerobic filters at different flow rates.

3.4. Performance of the SANI system

Since nitrogen removal was mainly achieved through autotrophic denitrification and nitrification in the anoxic and

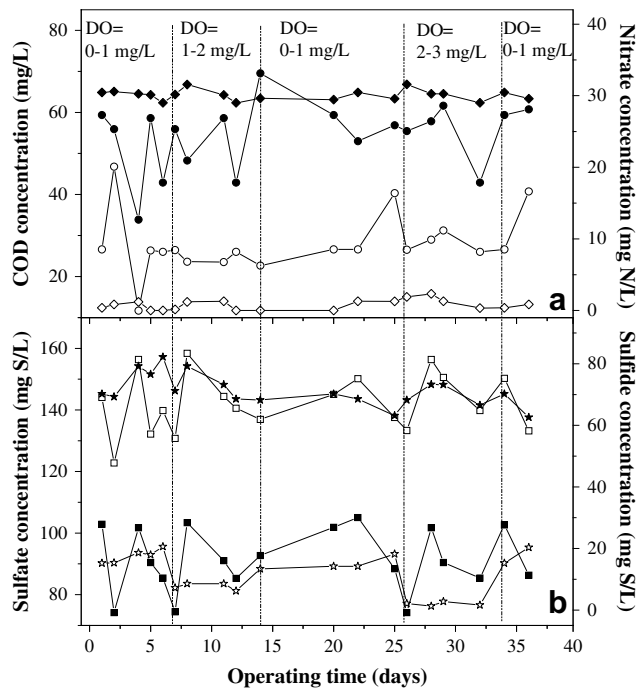


Fig. 3 – The performance of the anoxic filter with different influent DO levels: ●, influent COD; ○, effluent COD; ◆, influent NO₃-N; ◇, effluent NO₃-N; ■, influent sulfate; □, effluent sulfate; ★, influent sulfide; and ☆, effluent sulfide.

aerobic filters, respectively, the recirculation ratio (*R*) between the aerated and anoxic filters is the most important operational parameter in achieving a high total nitrogen removal by the system. Therefore, the effects of different recirculation ratios on the performance of the SANI system were mainly examined and the results are summarized in Table 3.

As shown in Table 3, the recirculation ratio variation had little influence on the COD removal of the SANI system. Nitrate reduction in the anoxic filter was almost complete when *R* was from 1 to 3*Q* (*Q* = influent flow rate of the system). However, when *R* was increased to 4*Q*, the nitrification and denitrification efficiencies were drastically reduced to 17 and 18%, respectively, resulting in a low total nitrogen removal of 35%. The reason of this reduction could be related to enhanced

oxygen input in the anoxic filter or the occurrence of a short-cut flow at the higher flow velocity.

The average total suspended solid (TSS) in the SANI system effluent was only 1.1 mg/L and no sludge was withdrawn from the system. All excess sludge produced was removed via the effluent, which was obviously a marginal amount.

4. Discussion

4.1. Competition of SRB and MPB in UASB for COD removal

The average COD-to-sulfate sulfur ratio in this study was 2.4. This suggested that about 83% COD reduction (as calculated from the theoretical value (2) to this measurement (2.4), i.e. $2/2.4 = 0.83$) came from SRB and the remaining COD was obviously removed through methanogenesis since MPB and SRB share many ecological and physiological similarities. Three general relationships between MPB and SRB are identified: (1) co-existence through using separate substrates; (2) a synergistic relationship in which one group of bacteria supplies the electron donors needed by the other to perform metabolic activities; and (3) competition for electron donors between them (Smith, 1993). However, from a thermodynamic point of view, the reduction of sulfate to sulfide by SRB yields more energy than methanogenesis bacteria, therefore enabling SRB to out-compete MPB. Furthermore, SRB are more competitive than MPB as SRB have higher affinity (or a lower K_s value) for limiting substrates (Khanal, 2002). This study confirmed that a higher COD-to-sulfate ratio in saline wastewater does not shift organic removal from SRB to MPB. In other words, provision of sufficient sulfide by SRB for autotrophic denitrification is not affected by excessively available COD in the UASB, as long as a minimum ratio of the sulfide sulfur consumed (*S*) to the nitrate nitrogen reduced (*N*) in the anoxic filter (*S/N* ratio) can be maintained.

4.2. Mixing effect on sulfate reduction in the UASB

An actual COD-to-sulfate ratio was influenced by the HRT or up-flow velocity and the internal recirculation ratio in UASB. Fig. 4 shows this ratio at different HRTs and internal

Table 3 – The performance of the SANI system under various recirculation ratios (*Q* is the influent flow rate of the system).

Parameters	I	II	III	IV
Anoxic filter/aerobic filter recirculation rate	1 <i>Q</i>	2 <i>Q</i>	3 <i>Q</i>	4 <i>Q</i>
Influent COD of anoxic filter (mg/L)	31.8 ± 1.5	15.5 ± 0.8	25.9 ± 1.3	21.4 ± 1.0
Effluent COD of aerobic filter or effluent of the system (mg/L)	14.1 ± 0.7	8.1 ± 0.4	14.7 ± 0.6	11.7 ± 0.6
TN influent of UASB (mg N/L)	30 ± 1.5	30 ± 1.3	30 ± 1.5	29 ± 1.5
TN effluent of aerobic filter (mg N/L)	16 ± 0.8	10 ± 0.5	8 ± 0.4	19 ± 0.9
Nitrate removal in anoxic filter (%) ^a	99 ± 4.1	99 ± 4.5	97 ± 4.6	8 ± 0.4
Nitrification efficiency in aerobic filter (%) ^b	98 ± 4.1	99 ± 4.5	93 ± 4.5	17 ± 0.8
TN removal efficiency (%) ^c	49 ± 2.4	65 ± 3.2	74 ± 3.7	35 ± 1.7
COD removal efficiency (%)	94.4 ± 4.7	96.9 ± 4.8	94.3 ± 4.7	94.2 ± 4.7

a Nitrate removal in anoxic filter(%) = NO₃ removed in anoxic filter(g N)/Influent NO₃ of anoxic filter(g N) × 100.

b Nitrification efficiency in aerobic filter(%) = NH₄ consumed in aerobic filter(g N)/Influent NH₄ of aerobic filter(g N) × 100.

c TN removal efficiency(%) = (Influent TN of the UASB – Effluent TN of the aerobic filter)/Influent TN of the UASB × 100.

recirculation ratios. When the UASB was operated with HRTs of 6, 4 and 3 h, respectively and the internal recycling ratio (IR) remained at 7, the COD-to-sulfate ratio varied between 0.73 and 0.82 (values of R^2 were more than 0.88). These results indicate that comparable sulfidogenic activities occurred under such different operating conditions. However, when the internal recycling ratio dropped to 5, the COD-to-sulfate ratio increased to 1.05, implying that there was a decrease in the electron flow to the sulfate-reducing pathways and the resulting COD removal by SRB decreased to 63%. This was most likely caused by insufficient internal mixing, which led to a poor substrate transfer of sulfate into sulfidogenic granules. After increasing this internal recycling ratio to improve the mixing conditions in the UASB, the COD-to-sulfate ratio and COD removal recovered to 0.75 and 89%, respectively. Sulfidogenic granules are generally expected to have an inferior mass transfer rate than methanogenic granules have (Lens et al., 1998) because less gaseous end products are formed. Higher production of gaseous products helps to eliminate the external diffusion resistance imposed by the stagnant liquid layer surrounding the granules (Huisman et al., 1990). This explains why organic removal through sulfate reduction was significantly affected by the mixing conditions in the UASB.

4.3. Competition of autotrophic and heterotrophic denitrification under sulfide-sufficient conditions

Since simultaneous autotrophic and heterotrophic denitrification is helpful in achieving economical nitrogen removal and reducing alkalinity consumption, interactions between

the autotrophic and heterotrophic denitrification reactions were evaluated under different organic loadings and alkalinity conditions in batch reactors or sulfur-packed beds (Kim and Bae, 2000; Oh et al., 2000, 2001; Kim et al., 2002). In most cases, a large portion of nitrate was removed heterotrophically and the remaining portion was denitrified by autotrophic denitrifiers without any inhibition in the presence of organic matter. This is probably attributable to the fact that the energy yield of autotrophic denitrification using elemental sulfur (91.5 kJ/electron equivalent) is somewhat lower than that of heterotrophic denitrification using methanol (109.18 kJ/electron equivalent) (Oh et al., 2003). However, at a lower organic loading, autotrophic denitrification in a sulfur-packed reactor was preferred, as indicated by an increased sulfate production and a shorter lag time. In our study, the average COD concentration in the influent of the anoxic filter was as low as 31 mg COD/L and the observed S/N ratio ranged from 1.47 to 2.23 with an average of 1.72 (values of R^2 were between 0.83 and 0.97) (see Figs. 5 and 6), which is close to a stoichiometric value of 1.93 using sulfide-S as the electron donor (Driscoll and Bisogni, 1978). This finding further demonstrates that, on average 89% nitrate (as calculated from $1.72/1.93 = 0.89$) was removed by autotrophic denitrification in the anoxic filter. The higher S/N ratio of 2.23 is close to the stoichiometric S/N ratio of 2.55 when elemental sulfur is used as the sulfur source (Koenig and Liu, 2001). This suggests that elemental sulfur might have been involved in the autotrophic denitrification as the sulfur source. The elemental sulfur possibly accumulated in the biomass and/or in the reactor during the start-up period when excessive thiosulfate was injected into the system.

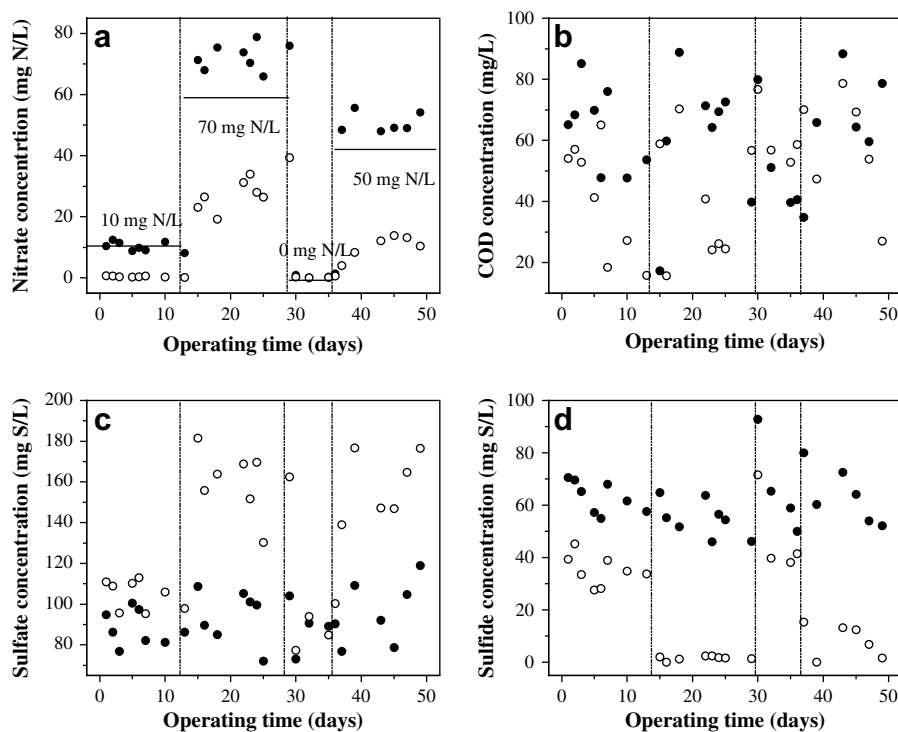


Fig. 4 – The performance of (a) nitrate removal, (b) COD removal, (c) sulfate regeneration, and (d) sulfide consumption under various nitrate concentrations. The solid and open symbols signify the influent and effluent of the anoxic filter, respectively.

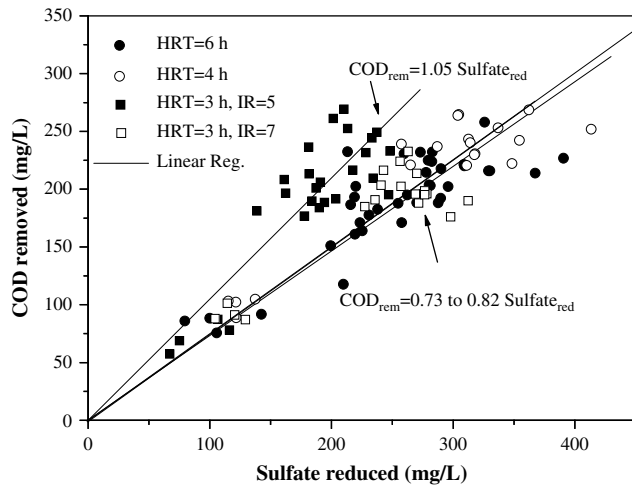


Fig. 5 – The correlation between COD removal and sulfate reduction in the UASB. (*UASB: HRT = 3 h, IR = 7; **UASB: HRT = 3 h, IR = 5).

4.4. Competition of autotrophic and heterotrophic denitrification under sulfide-limiting conditions

In the anoxic filter, the COD removal probably involved heterotrophic denitrification. It is impossible to estimate the exact amount of nitrate removed through heterotrophic denitrification. However, as shown in Tables 2 and 3, when the nitrate loading rate was adjusted to 0.36 kg N/m³-d in Run III, the observed S/N ratio decreased to an average of 1.21, showing that the amount of nitrate removed through autotrophic pathways was only 62.7%, while the total nitrate removal was 87.7%. This indicated that heterotrophic denitrification can make up for weak autotrophic denitrification under conditions of sulfur limitation. Even though heterotrophic denitrification would provide significant advantage in terms of nitrate and organic removal (Oh et al., 2001), higher excess sludge production over the long run occurs. This does not help us to meet our primary objective. In real applications, an HRT longer than 4 h in the UASB is advised to ensure better organic removal including particulate COD since a primary sedimentation tank would be removed to secure adequate production of sulfide in UASB, thereby lowering influent COD to the anoxic filter to limit the heterotrophic denitrification.

The performance of the anoxic filter reactor depends on the nitrogen loading rate, the HRT, and/or the mass transfer rate. As shown in Fig. 3, complete nitrate removal could not be achieved when the influent nitrate exceeded 50 mg N/L, due to sulfide limitation. This implied that the S/N ratio in the feed was highly correlated with the treatment performance. Fig. 7 presents the nitrate removal efficiency at different S/N ratios. When this ratio was lower the amount of sulfide became insufficient, causing lower nitrate removal. On the contrary, a complete nitrate removal was achieved when this ratio was greater than 2. Practically, a minimum S/N ratio of 1.6 is recommended to achieve more than 90% nitrate removal through autotrophic denitrification.

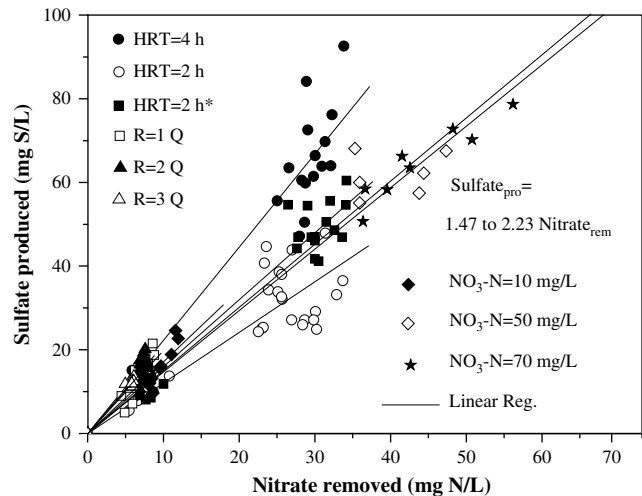


Fig. 6 – The correlation between sulfate production and nitrate removal in the anoxic filter.

4.5. Sulfur recovery

Table 4 shows the sulfur recovery efficiency in the UASB and the anoxic filter, respectively. The sulfur recovery efficiency in the UASB is based on the sulfate and dissolved sulfide, which varied from 80 to 93%. This confirmed that most of the influent sulfate ended up as dissolved sulfides, suggesting that the amount of hydrogen sulfide gas and the accumulation of elemental sulfur were trivial in the UASB. Meanwhile, the sulfur recovery efficiency in the anoxic filter was 91–99%, implying that there was almost complete oxidation of dissolved sulfide to sulfate. Hence, the loss of sulfur to elemental sulfur was trivial.

4.6. Sludge withdrawal

Over the entire period of this lab-scale study, there was not much difference in the effluent TSS in both of the USAB and the anoxic filter integrated setup and the complete SANI setup under different operating conditions. The average TSS

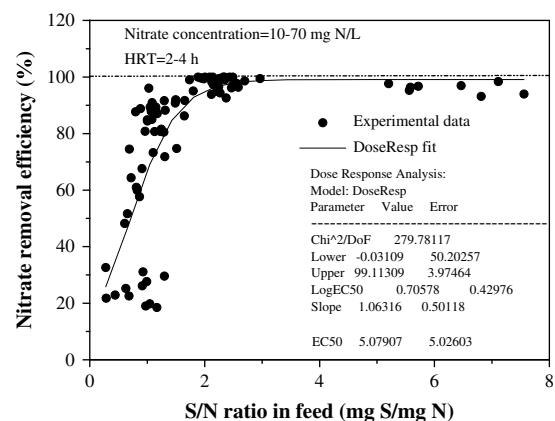


Fig. 7 – The effect of the S/N ratio of the feed on nitrate removal by the anoxic filter.

Table 4 – The sulfur balance for the UASB and anoxic filter of the SANI system under various operating conditions.

Reactor	Operating condition	Influent		Effluent		Sulfur recovery (%)
		SO ₄ ²⁻ (mg S/L)	HS ⁻ (mg S/L)	SO ₄ ²⁻ (mg S/L)	HS ⁻ (mg S/L)	
UASB	HRT = 3 h, IR = 7	183.2	/	96.3	73.1	92.5
	HRT = 3 h, IR = 5	183.5	/	116.5	49.2	90.3
	HRT = 4 h, IR = 5	185.2	/	78.4	87.9	89.8
	HRT = 6 h, IR = 4	165.0	/	71.7	60.7	80.2
Anoxic filter	HRT = 2 h ^a	87.09	66.45	136.57	11.73	97.54
	HRT = 2 h ^b	101.18	36.62	133.84	1.20	98.04
	HRT = 4 h	60.10	72.37	125.81	4.69	98.70
UASB	R ^c = 1Q	189.31	/	45.33	39.85	85.58
	R ^c = 2Q	178.74	/	45.94	45.73	91.65
	R ^c = 3Q	186.85	/	52.81	44.42	97.22
Anoxic filter	R ^c = 1Q	131.85	29.06	92.07	6.64	98.72
	R ^c = 2Q	133.14	22.95	95.04	4.14	99.10
	R ^c = 3Q	147.42	19.35	94.92	3.60	98.51

a UASB: HRT = 3 h, IR = 7.

b UASB: HRT = 3 h, IR = 5.

c Anoxic filter/aerobic filter recirculation rate (in Table 3).

concentrations in the effluent of the UASB, anoxic filter, and aerobic filter were 6.5, 4.5 and 1.1 mg/L, respectively. During the entire Part I study, no purposeful biomass withdrawal was conducted, evidently demonstrating the low sludge production in the SANI system. The MLVSS in the UASB was maintained at 6000 mg/L over the entire operation period (Tsang et al., in press; Lu et al., submitted for publication). The detailed reasons for this are reported in a theoretical evaluation of the SANI system (Lu et al., submitted for publication).

5. Conclusions

The lab-scale SANI process was successfully established to demonstrate the good potential for COD and nitrogen removal from saline wastewater with low sludge production. Experimental work lasted for 500 days to investigate the performance of the UASB, the anoxic filter and the entire SANI process on a laboratory scale under long-term stable process conditions. The main conclusions of this study are as follows.

- (1) The lab-scale SANI system successfully demonstrated high COD and nitrogen removal efficiencies (95% COD, 99% nitrate, and 74% TN) without withdrawal of sludge over a sufficiently long operating period with synthetic saline sewage. Average TSS concentrations in the effluent of the system were 1.1 mg/L.
- (2) A higher COD-to-sulfate ratio in saline wastewater did not affect sulfide production for autotrophic denitrification and more than 80% of COD was removed through sulfate reduction.
- (3) A minimum S/N ratio of 1.6 in the influent of the anoxic filter is necessary for achieving more than 90% nitrate removal through autotrophic denitrification, which is the major contributor to total nitrogen removal in the SANI system.
- (4) Sulfur balance analyses confirmed that accumulation of elementary sulfur and losses of hydrogen sulfide in the system were trivial.

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