



Effects of loading rate and hydraulic residence time on anoxic sulfide biooxidation*

CAI Jing, ZHENG Ping, MAHMOOD Qaisar, ISLAM Ejazul, HU Bao-lan^{†‡}, WU Dong-lei

(Department of Environmental Engineering, Zhejiang University, Hangzhou 310029, China)

[†]E-mail: blhu@zju.edu.cn

Received Dec. 4, 2006; revision accepted Mar. 12, 2007

Abstract: The optimal operation conditions in an anoxic sulfide oxidizing (ASO) bioreactor were investigated. The maximal removal rates for sulfide and nitrate were found to be 4.18 kg/(m³·d) and 1.73 kg/(m³·d), respectively. The volumetric loading rates (LRs) observed through decreasing hydraulic retention time (HRT) at fixed substrate concentration are higher than those by increasing substrate concentration at fixed HRT. The sulfide oxidation in ASO reactor was partially producing both sulfate and sulfur, but the amount of sulfate produced was approximately one third that of sulfur. The process was able to tolerate high sulfide concentration, as the sulfide removal percentage always remained near 99% when influent concentration was up to 580 mg/L. It tolerated relatively lower nitrate concentration because the removal percentage dropped to 85% when influent concentration was increased above 110 mg/L. The process can tolerate shorter HRT but careful operation is needed. Nitrate conversion was more sensitive to HRT than sulfide conversion since the process performance deteriorated abruptly when HRT was decreased from 3.12 h to 2.88 h. In order to avoid nitrite accumulation in the reactor, the influent sulfide and nitrate concentrations should be kept at 280 mg/L and 67.5 mg/L respectively. Present biotechnology is useful for removing sulfides from sewers and crude oil.

Key words: Anaerobic processes, Anoxic nitrate removal, Biotransformation, Fluidized bed bioreactors, Hydraulic residence time (HRT)

doi:10.1631/jzus.2007.A1149

Document code: A

CLC number: X703.1

INTRODUCTION

Biotechnology can be employed in assessing the well being of ecosystems, transformation of pollutants into benign substances, generation of biodegradable materials from renewable sources, and developing environmentally safe manufacturing and disposal processes. Environmental biotechnology employs microbial metabolism to reduce the environmental burden of toxic substances.

Sulfide rich waste streams are generated by a number of industries such as petrochemical plants, tanneries, viscose rayon factories, and the processes of the gasification of coal for electricity production,

or the anaerobic treatment of sulfate containing wastewaters. Various toxicological effects of sulfide on human health have been described elsewhere (Jappinen *et al.*, 1990; Bhambhani and Singh, 1991; Kilburn and Warshaw, 1995). Large amounts of wastewaters are generated from industry, agriculture and housing settlements containing nitrogenous compounds. Untreated discharge of these wastewaters to the environment can lead to serious environmental problems. Nitrate or nitrite can induce multi-dimensional hazards, such as the eutrophication of water bodies (Zheng *et al.*, 2004).

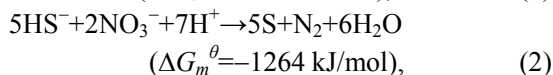
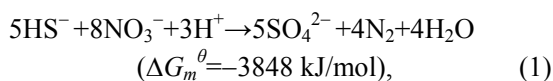
A wide range of physico-chemical processes such as ion exchange, reverse osmosis, electro dialysis, chemical and biological denitrification has been developed currently for the removal of nitrate/nitrite from wastewater (Kapoor and Viraraghavan, 1997). Being economic and efficient, biological process has

[‡] Corresponding author

* Project supported by the National Natural Science Foundation of China (No. 30070017) and the Science and Technology Foundation for Key Project of Zhejiang Province (No. 2003C13005), China

been the most popular technology for wastewater treatment. It has been shown that some bacterial species like *Thiobacillus denitrificans* can oxidize sulfide to elemental sulfur simultaneously reducing nitrate to dinitrogen (Krishnakumar and Manilal, 1999). Because autotrophic denitrifiers utilize inorganic carbon compounds (such as CO_2 , HCO_3^-) as their carbon source (Claus and Kutzner, 1985; Gayle *et al.*, 1989), no organic carbon is needed as in heterotrophic denitrification implying less sludge production and thereby minimization of the excessive sludge disposal (Claus and Kutzner, 1985; Koenig and Liu, 1996; Zhang and Lampe, 1999). The anoxic sulfide oxidizing (ASO) process can also save the energy for aeration since the bacteria use nitrate instead of oxygen as electron acceptor. For such reasons, the ASO process has received much attention recently.

At the optimum pH value of 7.5 (Krishnakumar and Manilal, 1999), the sulfide exists in the form of HS^- . During the ASO process, the actual chemical reactions are as follows:



where ΔG_m^θ is standard free energy.

From the chemical Eqs.(1) and (2), it is obvious that the sulfide to nitrate ratio in the influent should be a key factor.

The objectives of this study were to assess the potential of the ASO process and to determine the optimal operational conditions.

MATERIALS AND METHODS

ASO reactor

The ASO reactor is an up flow reactor with biomass retention in a continuous mode of operation. Its schematic diagram is shown in Fig.1. The reactor is made of perspex with a working volume of 1.3 L. The synthetic influent is pumped through a peristaltic pump from the 5-L influent vessel to the reactor. The flow rate can vary between 0.6 to 12.5 L/d, which gives the possibility to operate at hydraulic residence time (HRT) between 2 and 0.1 d. A recycling pump is

used to mix the influent (substrate) and sludge (biocatalyst) well and hence to decrease possible substrate inhibition. The ratio of recycling flow to the influent flow was set to about 2.5~3. The temperature of the reactor can be controlled between 20 °C and 70 °C using a thermostat, although the normal operational temperature is 30 °C.

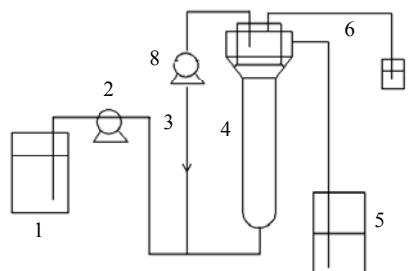


Fig.1 The schematic presentation of experimental set up

1: Influent tank; 2: Feeding pump; 3: Recycling port; 4: ASO reactor; 5: Effluent container; 6: Port to gas collector; 7: Gas collector containing water; 8: Recycling pump

Inoculum and enrichment of microbial communities

Inoculum was taken from the anaerobic methanogenic reactor in Dengta Wastewater Treatment Plant (WWTP) located in Hangzhou City of China. Its total solids (TS) and volatile suspended solids (VSS) were 95.03 g/L and 68.68 g/L respectively, with VS/TS ratio of 0.72. The ASO reactor was operated under lithoautotrophic conditions where sulfide was used as electron donor and nitrate as electron acceptor to accomplish denitrification. For the initial one month the reactor was fed with synthetic wastewater in order to enrich the sludge and for acclimatization of bacteria to the new substrates.

Synthetic wastewater

The reactor was fed with synthetic influent containing NaHCO_3 , MgCl_2 , KH_2PO_4 (1 g/L each), $(\text{NH}_4)_2\text{SO}_4$ (0.24 g/L) and trace element solution (1 ml/L). The composition of trace element solution was used according to (Mahmood *et al.*, 2007). The nitrate-nitrogen and sulfide-sulfur concentrations were added in the form of sodium sulfide ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) and potassium nitrate (KNO_3), respectively with their concentrations varying according to the type of experiment conducted. The used influent sulfide to nitrate ratio was 5:2 while assessing the potential of ASO bioreactor.

Operational parameters

Operational and performance parameters used for ASO reactor include volumetric loading rates, HRT and removal efficiency (RE) (Mahmood *et al.*, 2007). Mass loading rate defines the amount of contaminant entering the ASO reactor per unit volume per unit time. HRT is the time that a unit volume of wastewater will remain in ASO reactor and overestimates the actual treatment time. RE is used to describe the performance of ASO reactor. As the loading rate is increased at fixed HRT, a point of saturation or the maximum RE corresponding to maximum microbial substrate utilization rate is observed. At fixed substrate concentration with decreasing HRT determines the minimum time period for maximum treatment efficiency. RE is the fraction of contaminant removed by bioreactor. RE is an incomplete descriptor of bioreactor performance because it varies with contaminant concentration, flow rate and bioreactor size and reflects only the specific conditions under which it is measured (Mahmood *et al.*, 2007).

Analytical procedures

Ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen, pH, sulfide and sulfate were determined (both from influent and effluent) during the operation of ASO reactor. Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) was measured by Phenate method (APHA, 1998), nitrite

nitrogen ($\text{NO}_2^-\text{-N}$) through colorimetric method and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) was analyzed through ultraviolet spectrophotometric screening method (APHA, 1998), nitrite nitrogen ($\text{NO}_2^-\text{-N}$) through colorimetric method and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) was analyzed through ultraviolet spectrophotometric screening method (APHA, 1998) on daily basis using spectrophotometer (Unico UV-2102 PC and 722S, China). The sulfide was determined by iodometric method and sulfate was measured through turbidimetric method (APHA, 1998). The pH was determined according to Standard Method (APHA, 1998). A three-point calibration of pH meter was performed daily. TS concentrations were determined according to gravimetric method at 103 °C (APHA, 1998) and volatile solids (VS) were analyzed through gravimetric method at 550 °C (APHA, 1998).

Statistical and graphical work

Statistical work was carried out by using Microsoft Excel while computer program Sigma Plot V.10 was used to carry out graphical work.

RESULTS AND DISCUSSIONS

Results

During start up of ASO reactor, the concentra-

Table 1 Performance of the ASO reactor during start-up

Time (d)	HRT (d)	Sulfide-sulfur				Nitrate-nitrogen				Nitrogen formed	$Y_{\text{N}_2}^c$
		IC	EC	RE ^a	LR ^b	IC	EC	RE ^a	LR ^b		
1	2.19	114.05	2.00	98.24	0.127	41.00	8.60	79.02	0.048	32.40	0.79
		±2.36	±0.32	±0.32	±0.004	±1.21	±0.60	±2.03	±0.05	±2.11	±0.08
5	1.37	176.15	1.36	99.02	0.159	53.56	9.70	86.43	0.068	43.86	0.81
		±5.48	±0.14	±0.31	±0.005	±6.84	±1.16	±0.57	±0.001	±4.64	±0.09
9	1.80	191.51	0.83	99.54	0.325	60.48	10.96	82.47	0.103	49.52	0.81
		±10.0	±0.21	±0.15	±0.008	±1.02	±1.45	±2.07	±0.001	±2.73	±0.05
16	1.13	236.40	0.96	99.59	0.406	68.96	7.86	88.95	0.117	61.10	0.88
		±9.73	±0.07	±0.05	±0.008	±1.76	±0.66	±0.87	±0.007	±3.22	±0.05
20	0.90	255.40	1.45	99.46	0.568	82.50	24.9	71.08	0.190	57.60	0.69
		±12.60	±0.09	±0.03	±0.010	±4.12	±3.22	±3.89	±0.003	±5.29	±0.06
23	0.81	260.04	1.12	99.57	0.679	72.20	20.57	73.07	0.194	51.63	0.71
		±13.87	±0.13	±0.09	±0.012	±3.45	±5.38	±6.85	±0.005	±4.39	±0.06
26	0.75	264.09	1.56	99.42	0.767	76.07	21.47	72.39	0.232	54.60	0.71
		±13.09	±0.62	±0.24	±0.011	±6.06	±4.36	±5.78	±0.004	±6.21	±0.08

RE: removal efficiency (%); LR: loading rate [$\text{kg}/(\text{m}^3\cdot\text{d})$]; IC: influent concentration (mg/L); EC: effluent concentration (mg/L); Y_{N_2} : denitrifying yield ($\text{mg N}_2/\text{mg NO}_3\text{-N}$); ^aRE (%) = $(c_{\text{in}} - c_{\text{fin}}) \cdot 100 / c_{\text{in}}$, where c_{in} is the concentration of pollutant in the influent and c_{fin} is its concentration in the effluent; ^bLR is the amount of contaminant entering the ASO reactor per unit volume per unit time; ^c Y_{N_2} is the amount of dinitrogen produced per mg nitrate nitrogen utilized in the ASO reactor (Note that the minor product such as nitrite can be ignored, because it is always less than 0.1 mg/L)

tions of nitrate-nitrogen and sulfide-sulfur in the influent were increased from 40 mg/L to 69 mg/L and from 114 mg/L to 236 mg/L respectively, at the same time HRT was decreased stepwise from 2.19 d to 1.37 d (Table 1). The removal percentages of nitrate-nitrogen and sulfide-sulfur in ASO reactor were very high as can be seen in Table 1. The nitrate removal percentage remained higher than 80%, while sulfide removal percentage was approximately 99.5%. For the next 17~26 d, the concentrations of nitrate-nitrogen and sulfide-sulfur in the influent were kept increasing till 76.07 mg/L and 264.09 mg/L respectively. The removal percentages of nitrate-nitrogen and sulfide-sulfur were about 72.39% and 99.42% respectively. The volumetric loading rates of nitrate and sulfide were 0.071 kg/(m³·d) and 0.323 kg/(m³·d) respectively. The sulfide-sulfur in the effluent was lower than 2 mg/L (Table 1). After operating 26 d the reactor reached steady state, the criteria for which were sulfide and nitrate removal rates of over 95% and 70%, respectively. As these removal percentages remained stable for at least 15 d, these were considered as steady state criteria.

1. Potential of the ASO reactor

Volumetric loading rate is an important index to assess the potential of a bioreactor. At fixed HRT of 0.5 d, the influent sulfide-sulfur concentration was kept on increasing from 100 mg S/L to 580 mg S/L, the sulfide-sulfur volumetric loading rate ranged from 0.67 to 3.29 kg/(m³·d) while the RE was maintained higher than 90% (Fig.2). Meanwhile, the sulfide-sulfur RE reached 3.01 kg/(m³·d).

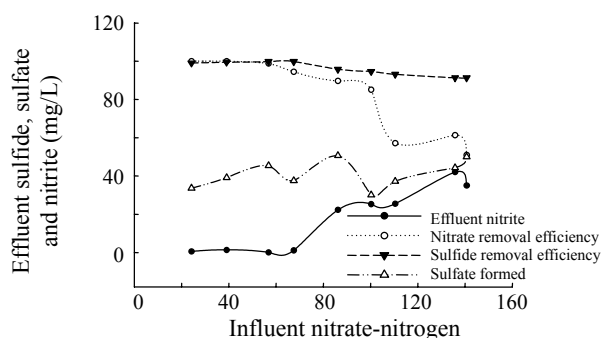


Fig.2 Steady state performance of ASO reactor during loading tests at fixed HRT (0.5 d)

During HRT tests at fixed influent sulfide concentration with decreasing HRT from 1 d to 0.12 d, the sulfide-sulfur volumetric loading rate ranged from

0.52 kg/(m³·d) to 4.18 kg/(m³·d). The RE was maintained higher than 90% with effluent sulfide concentration of less than 2 mg/L during HRT tests (Fig.3).

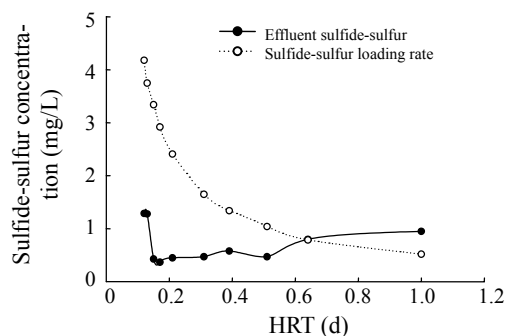


Fig.3 Effect of HRT on sulfide oxidation

At fixed HRT of 0.5 d, with increasing influent nitrate-nitrogen concentration from 24.2 to 140.7 mg/L, volumetric loading rate ranged from 0.16~0.81 kg/(m³·d) with RE of 50.89%~100% (Fig.2). Keeping influent sulfide concentration constant, with decreasing HRT from 1 d to 0.12 d, the nitrate-nitrogen volumetric loading rate was in the range of 0.12~1.73 kg/(m³·d), while the RE ranged from 92% to 58.78%, and the nitrate-nitrogen volumetric removal rate was in the range of 0.12~1.02 kg/(m³·d) (Fig.4).

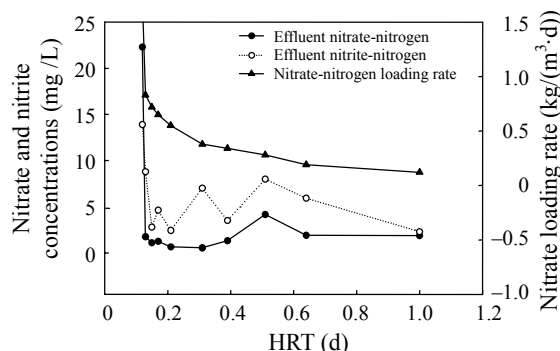


Fig.4 Effect of HRT on nitrate reduction in ASO reactor

2. Effect of HRT

During the experiment, HRT had little impact on sulfide removal percentage. As HRT was decreased from 1 d to 0.12 d, the effluent sulfide-sulfur concentration remained lower than 1.29 mg/L, and RE was always kept higher than 99%. The optimum HRT for sulfide conversion was as low as 0.12 d (Fig.3).

HRT had a notable impact on nitrate removal percentage for the tested range. When HRT was de-

creased from 1 d to 0.13 d, nitrate RE always remained higher than 92%. However, as HRT was decreased from 0.13 d to 0.12 day, effluent nitrate concentration rose to 22.3 mg/L, and RE dropped to 58.78%; at the same time the effluent nitrite concentration increased from 8.79 mg/L to 13.90 mg/L (Fig.4). So, the optimum HRT for nitrate conversion was about 0.13 d.

3. Effect of pH

Effluent pH is a good stability indicator of reactor performance. The effluent pH was plotted against sulfide and nitrate removal efficiencies observed during volumetric loading tests (Fig.5). It was obvious that the sulfide and nitrate removal was at its optimum in pH range of 7.6~8.1. As the sulfide-sulfur and nitrate-nitrogen volumetric loading was increased, the effluent pH also increased consequently resulting in a drop of sulfide and nitrate removal. Finally the process deteriorated at the effluent pH value of 9.2. Nitrate removal was found more sensitive to pH change compared with sulfide oxidation.

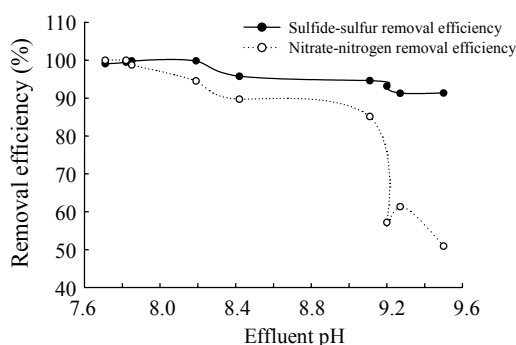


Fig.5 Relation between pH and ASO reactor performance during volumetric loading tests

Discussion

The present investigation was carried out using 5:2 sulfides to nitrate ratios aiming to get elemental sulfur as major oxidation product. Both sulfate and sulfur were produced due to partial oxidation during the present investigation. Our findings are quite consistent with earlier researches (Yang and Allen 1994a; 1994b; Sublette and Heskth, 1994; Chung *et al.*, 1996; Zhang and Lampe, 1999; Kleerebezem and Mendez, 2002; Vaiopoulou *et al.*, 2005).

Higher sulfide volumetric loading rates (i.e. 4.18 kg/(m³·d) were achieved in the present investigation during HRT compared with earlier studies utilizing

chemotrophic systems for treatment of various kinds of wastewaters (Kobayashi *et al.*, 1983; Buisman and Lettinga, 1990; Lee and Sublette, 1993; Buisman *et al.*, 1993a; 1993b; Basu *et al.*, 1996; Khanna *et al.*, 1996; Janssen *et al.*, 1997). According to the earlier investigations, the sulfide volumetric loading rate for biological treatment was 0.042~0.294 kg/(m³·d) (Stefess and Yebeeb, 1989; Buisman, *et al.*, 1990; 1991; Buisman and Lettinga, 1990), while the maximum nitrate loading rate for biological denitrification was 0.175~0.594 kg/(m³·d) (Chen *et al.*, 1995; Koenig and Liu, 1997). Though Janssen *et al.* (1997) obtained 14 kg/(m³·d) sulfide loading rate while treating anaerobic effluents from sulfur-sludge; however their inlet sulfide concentration was lower (240 mg/L) compared with the present work. Moreover their sulfide RE was 90% whereas steady state sulfide RE during present study was always above 99%. During present study high inlet sulfide-sulfur volumetric loading rates ranging 100~540 mg/L were treated effectively in mixed microbial culture which have not been achieved by previous studies reported above (Buisman and Lettinga, 1990; Buisman *et al.*, 1993a; 1993b; Lee and Sublette, 1993; Janssen *et al.*, 1997; Reyes-Avila *et al.*, 2004).

The influent sulfide concentration had little influence on sulfide removal percentage in the range of 100~580 mg/L and the reactor worked smoothly. As sulfide concentration was increased, the effluent sulfide concentration went up slightly, but removal percentage always remained higher than 90%. Judged by the sulfide conversion, the optimum sulfide concentration might possibly be higher than 580 mg/L (Fig.2). There was no detectable effluent nitrate concentration with the increasing influent nitrate concentration, from 24.2~39.1 mg/L, as removal percentage was 100%. But as the influent nitrate-nitrogen concentration was increased to 140.7 mg/L, the nitrate removal percentage decreased to 50.89%, with effluent nitrate concentration of 69.1 mg/L. When influent nitrate-nitrogen concentration reached 67.5 mg/L, nitrite began to accumulate in the reactor. Also, the performance of the reactor was not as stable as before. According to the nitrate conversion and the nitrite accumulation, the optimum nitrate-nitrogen concentration is about 70 mg/L (Fig.6).

Mass balance analysis was carried out to underpin the nature of biochemical reactions occurring in

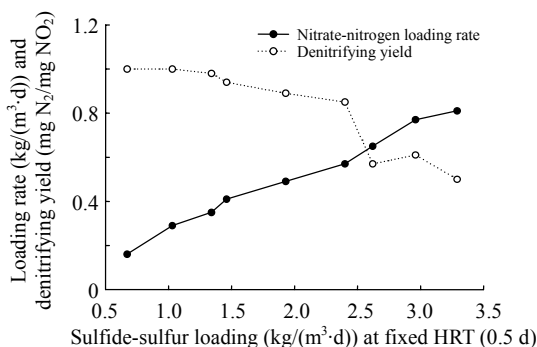


Fig.6 Steady state reactor performance at fixed HRT (0.5 d)

the bioreactor. Eq.(2) shows the sulfide and nitrate reacted at S:N ratio of 5:2. According to Eq.(2) each mg of sulfide (as HS^-) oxidized can produce 0.97 mg of sulfur and 0.17 mg of N_2 ; while each mg of nitrate reduced can contribute to 1.29 mg of sulfur and 0.225 mg of N_2 . During loading tests, at sulfide-sulfur concentration of 100 mg/L produced an effluent of 0.96 mg/L implying that 99.04 mg sulfide was involved in sulfur formation. Hence the expected sulfide formation was $99.04 \times 0.97 = 96$ mg/L. However some amount of sulfate detected in the effluent was 33.62 mg/L implying that due to partial sulfide oxidation 35% sulfide might have been converted to sulfate while about 65% sulfide was converted to sulfur. Likewise the expected sulfur formation at influent sulfide-sulfur concentration of 580 mg/L can be evaluated based on the principle of stoichiometry. About 50 mg/L sulfate was detected at 580 mg/L sulfide-sulfur concentration. It can be assumed logically that the rest of the sulfide must have been oxidized to elemental sulfur.

The contribution of nitrate-nitrogen towards sulfide oxidation can also be analyzed. Eq.(2) shows that each mg of nitrate-nitrogen reduced could produce 1.29 mg of sulfur. At 100.25 mg/L influent nitrate-nitrogen, the amount of effluent nitrate was 14.9 mg/L thus 85.35 mg/L contributed for sulfide oxidation. Expected amount of sulfur should be 110.10 mg/L; while 30.09 mg sulfate was detected in the effluent (Fig.2). It means that the rest of the sulfide must have been converted to elemental sulfur in the bioreactor. Likewise analysis of 140 mg/L influent nitrate-nitrogen would result in the production of 180 mg/L sulfur based on stoichiometry of reaction Eq.(2). At 140 mg/L influent nitrate-nitrogen concentration,

sulfate observed was 50 mg/L suggesting a partial oxidation. Denitrifying yield of ASO reactor has been presented in Fig.6 at various sulfide and nitrate loadings at fixed HRT of 0.5 d. It shows that denitrifying yield decreased sharply over sulfide-sulfur and nitrate-nitrogen loadings of 2.5 and 0.6 $\text{kg}/(\text{m}^3 \cdot \text{d})$ respectively. Similar findings of partial sulfide oxidation were obtained during HRT experiment.

During studies on biooxidation of sulfide by pure culture of *Thiomicrospira* sp., Gadekar *et al.*(2006) established that sulfate formation was related to the sulfide to nitrate concentrations. Lower values of sulfide to nitrate ratio resulted in sulfate as the main product of sulfide biooxidation while increasing this ratio produced very much less amount of sulfate. Our results accord with earlier investigations regarding the effects of influent sulfide to nitrate ratios during biooxidation of sulfide. Janssen *et al.*(1997) observed that lower sulfide to oxygen ratios resulted in the production of both sulfur and sulfate, while under oxygen limited conditions thiosulfate was the main product.

Wang *et al.*(2005) showed that both sulfide concentration and the inlet sulfide to nitrate ratios were determinant of sulfide oxidation. The desirable levels for achieving sulfur as main product were proposed to be less than 9 mmol/L sulfide and inlet sulfide to nitrate ratios ranging 1.6~2.5, respectively. The present investigation was carried out using inlet sulfide to nitrate ratios of 2.5. As the influent sulfide was increased at fixed sulfide-sulfur to nitrate-nitrogen ratios and HRT, partial sulfide oxidation occurred resulting in sulfur as major biooxidation product which was indicated by the white color of the sludge (Krishnakumar and Manilal, 1999). Organic sulfur in cells can be estimated by measuring volatile solids as sulfur comprises 1% of dry cell weight (Shuler and Kargi, 2002). However, it was not possible to measure volatile solids frequently due to the very slow growth rate of bacterial communities and thus smaller amount of sludge. The effects of different sulfide to nitrate ratios in ASO reactor should be investigated further to underpin the exact mechanism of the process.

At fixed HRT toxic substrate increments did not inhibit the substrate utilization. The concentration of sulfide and nitrate did not increase in the reactor due to efficient utilization by microbial biomass in the

reactor. At lower concentrations the reaction rate increases with an increase in substrate (Rittmann and McCarty, 2002). However, when maximum rate is reached beyond which substrate concentrations become inhibitory, causing a decrease in reaction rate. Such kind of inhibition is called as self-inhibition, which is also called as Haldane or Andrews's kinetics (Rittmann and McCarty, 2002). Sudden drop in the ASO reactor performance utilizing nitrate might be due to self-inhibition.

At fixed substrate concentration with decreasing HRTs, the reactor can tolerate shorter HRTs with high influent volumetric loading rates if specific growth rate of microorganisms under the conditions is greater than HRT applied. However, if the influents are highly concentrated containing high volumetric loading rates at shorter HRTs, the specific growth rate of bacteria can be inhibited resulting in the building up of toxic substrates in the reactor. As ASO reactor with nitrate as electron acceptor tolerated shorter HRT of about 3 h (0.13 d), bacterial populations involved will not be able reproduce faster enough than they are removed from the reactor. The reactor population would continue to increase and substrate concentrations continue to decrease until the microbial population size and substrate concentrations reach their steady state conditions. The present biotechnology can be applied to treat sulfides of crude oil and sewers.

CONCLUSION

The sulfide oxidation in ASO reactor was partially producing both sulfate and sulfur; but the amount of sulfate produced was approximately one third that of sulfur. The process is capable of tolerating high influent substrate concentration i.e. 580 mg/L sulfide-sulfur and 140.7 mg/L nitrate-nitrogen with sulfide-sulfur and nitrate-nitrogen volumetric loading rates of 4.18 kg/(m³·d) and 1.73 kg/(m³·d) respectively at 0.13 d HRT. The reactor can tolerate higher loading rate at shorter HRT compared with longer HRT. The process can tolerate lower HRT but careful operation is needed while decreasing HRT. At HRT of 0.13 d, sulfide-sulfur and nitrate-nitrogen removal efficiencies were always maintained higher than 99% and 92% respectively. Nitrate conversion is more sensitive to HRT than sulfide conversion since the

process performance deteriorated abruptly when HRT was decreased from 0.13 d to 0.12 d. The process can tolerate relatively lower nitrate concentration. The removal percentage dropped to 85% when the influent nitrate-nitrogen concentration was higher than 110 mg/L. In order to avoid nitrite accumulation in the reactor, the influent sulfide-sulfur and nitrate-nitrogen concentrations should be kept at 280 mg/L and 67.5 mg/L respectively.

References

- APHA (American Public Health Association, Inc.), 1998. Standard Methods for the Examination of Water and Wastewater (20th Ed.). New York, USA.
- Basu, R., Clausen, E.C., Gaddy, J.L., 1996. Biological conversion of hydrogen sulfide into elemental sulfur. *Environ. Prog.*, **15**(4):234-238. [doi:10.1002/ep.670150412]
- Bhambhani, Y., Singh, M., 1991. Physiological effects of hydrogen sulfide inhalation during exercise in healthy men. *Appl. Physiol.*, **71**:1872-1877.
- Buisman, C.J.N., Lettinga, G., 1990. Sulfide from anaerobic waste treatment effluent of a paper mill. *Wat. Res.*, **24**(3):313-319. [doi:10.1016/0043-1354(90)90006-R]
- Buisman, C.J.N., Bert, G., Ijspeert, P., 1990. Optimization of sulfur production in biotechnological sulfide-removing reactor. *Biotechnol. Bioeng.*, **35**(1):50-56. [doi:10.1002/bit.260350108]
- Buisman, C.J.N., Ijspeert, P., Lettinga, G., 1991. Kinetic parameters of a mixed culture oxidizing sulfide and sulfur with oxygen. *Biotechnol. Bioeng.*, **38**(8):813-821. [doi:10.1002/bit.260380803]
- Buisman, C.J.N., Boer, J.D., Boonstra, J., 1993a. A New Biotechnological Method for H₂S Removal from Biogas. TAAPI Proceedings, p.773.
- Buisman, C.J.N., Bloembergen, J.R., Paalvast, C., 1993b. Biological Sulfur Recovery from Paper Mill Effluent. TAAPI Proceedings, p.841.
- Chen, F.G., Li, W.G., Pan, G.M., Liu, J.X., Jin, C.J., 1995. The study of nitrogen removal with anoxic-aerobic biomembrane process. *China Environ. Sci.*, **15**(2): 135-138.
- Chung, Y.C., Huang, C., Tseng, C.P., 1996. Operation optimization of *Thiobacillus thioparus* CH11 biofilter for hydrogen sulfide removal. *J. Biotechnol.*, **52**(1):31-38. [doi:10.1016/S0168-1656(96)01622-7]
- Claus, G., Kutzner, H.J., 1985. Autotrophic denitrification by *Thiobacillus denitrificans* in a packed bed reactor. *Appl. Microbiol. Biotechnol.*, **22**(4):289-296. [doi:10.1007/BF00252032]
- Gadekar, S., Nemati, M., Hill, G.A., 2006. Batch and continuous biooxidation of sulfide by *Thiomicrospira* sp. CVO: Reaction kinetics and stoichiometry. *Wat. Res.*, **40**(12):2436-2446. [doi:10.1016/j.watres.2006.04.007]
- Gayle, B.P., Boardman, G.D., Sherreard, J.H., Benoit, R.E., 1989. Biological denitrification of water. *J. Environ. Eng.*,

- 115(5):930-943.
- Janssen, A.J.H., Ma, S.C., Lens, P., 1997. Performance of a sulfide oxidizing expanded bed reactor supplied with dissolved oxygen. *Biotechnol. Bioeng.*, **53**(1):32-40. [doi:10.1002/(SICI)1097-0290(19970105)53:1<32::AID-BT6>3.0.CO;2-#]
- Jappinen, P., Vilkkä, V., Marttila, O., 1990. Exposure to hydrogen sulfide and respiratory function. *Br. Ind. Med.*, **47**:824-828.
- Kapoor, A., Viraraghavan, T., 1997. Nitrate removal from drinking water—Review. *J. Environ. Eng.*, **123**(4):371-380. [doi:10.1061/(ASCE)0733-9372(1997)123:4(371)]
- Khanna, P., Rajkumar, B., Jyothikumar, N., 1996. Microbial recovery of sulfur from thiosulfate bearing wastewater with phototrophic and sulfate reducing bacteria. *Current Microbiol.*, **32**(1):33-37. [doi:10.1007/s002849900006]
- Kilburn, K.H., Warshaw, R.H., 1995. Hydrogen sulfide and reduced-sulfur gases adversely affect neurophysiological functions. *Toxicol. Ind. Health*, **11**:185-197.
- Kleerebezem, R., Mendez, R., 2002. Autotrophic denitrification for combined hydrogen sulfide removal from biogas and post-denitrification. *Wat. Sci. Tech.*, **45**(10):349-356.
- Kobayashi, A.H., Stenstrom, M., Mah, R.A., 1983. Use of photosynthetic bacteria for hydrogen sulfide removal from anaerobic waste treatment effluent. *Wat. Res.*, **17**(5):579-587. [doi:10.1016/0043-1354(83)90117-3]
- Koenig, A., Liu, L.H., 1996. Autotrophic denitrification of landfill leachate using elemental sulphur. *Wat. Sci. Tech.*, **34**(5-6):469-476. [doi:10.1016/0273-1223(96)00680-4]
- Koenig, A., Liu, L.H., 1997. The study of landfill leachate treatment by *Thiobacillus denitrificans*. *J. Environ. Sci.*, **18**:51-54.
- Krishnakumar, B., Manilal, V.B., 1999. Bacterial oxidation of sulphide under denitrifying conditions. *Biotechnology Letters*, **21**(5):437-440. [doi:10.1023/A:1005584920800]
- Lee, M.C., Sublette, K.L., 1993. Microbial treatment of sulfide-laden water. *Wat. Res.*, **27**(5):839-846. [doi:10.1016/0043-1354(93)90148-B]
- Mahmood, Q., Zheng, P., Cai, J., Wu, D.L., Hu, B.L., Li, J.Y., 2007. Anoxic sulfide biooxidation using nitrite as electron acceptor. *J. Hazard. Mater.* (in Press). [doi:10.1016/j.jhazmat.2007.01.002]
- Reyes-Avila, J., Razo-Flores, E., Gomez, J., 2004. Simultaneous biological removal of nitrogen, carbon and sulfur by denitrification. *Wat. Res.*, **38**(14-15):3313-3321. [doi:10.1016/j.watres.2004.04.035]
- Rittmann, B.E., McCarty, P.L., 2002. Environmental Biotechnology: Principles and Applications. McGraw-Hill Companies, Inc., p.502.
- Shuler, M.L., Kargi, F., 2002. Bioprocess Engineering, Basic Concepts (Second Ed.). Prentice-Hall Inc., Englewood Cliffs, NJ, p.158-160.
- Stefess, G.C., Yebe, J.G., 1989. Factors influencing elemental sulfur production from sulfide or thiosulfate by autotrophic *Thiobacilli*. *Forum Microbiology*, **12**:92-101.
- Sublette, K.L., Hesketh, R.P., 1994. Microbial oxidation of hydrogen sulfide in a pilot-scale bubble column. *Biotechnology Progress*, **10**(6):611-614. [doi:10.1021/bp00030a005]
- Vaiopoulou, E., Melidis, P., Aivasidis, A., 2005. Sulfide removal in wastewater from petrochemical industries by autotrophic denitrification. *Wat. Res.*, **39**(17):4101-4109. [doi:10.1016/j.watres.2005.07.022]
- Wang, A.J., Du, D.Z., Ren, N.Q., 2005. An innovative process of simultaneous desulfurization and denitrification by *Thiobacillus denitrificans*. *J. Environ. Sci. Health*, **40**:1939-1949.
- Yang, Y., Allen, E.R., 1994a. Biofiltration control of hydrogen sulfide. 1. Design and operational parameters. *Journal of Air and Waste Management Association*, **44**:863-868.
- Yang, Y., Allen, E.R., 1994b. biofiltration control of hydrogen sulfide. 2. Kinetics, biofilter performance and maintenance. *Journal of Air and Waste Management Association*, **44**:1315-1321.
- Zhang, T.C., Lampe, D.G., 1999. Sulfur: Limestone autotrophic denitrification processes for treatment of nitrate-contaminated water: Batch experiments. *Wat. Res.*, **33**(3):599-608. [doi:10.1016/S0043-1354(98)00281-4]
- Zheng, P., Xu, X.Y., Hu, B.L., 2004. New Theory and Technology for Biological Nitrogen Removal. Beijing Science Press, Beijing (in Chinese).