

Anoxic sulfide biooxidation using nitrite as electron acceptor

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Abstract

Biotechnology can be used to assess the well being of ecosystems, transform pollutants into benign substances, generate biodegradable materials from renewable sources, and develop environmentally safe manufacturing and disposal processes. Simultaneous elimination of sulfide and nitrite from synthetic wastewaters was investigated using a bioreactor. A laboratory scale anoxic sulfide-oxidizing (ASO) reactor was operated for 135 days to evaluate the potential for volumetric loading rates, effect of hydraulic retention time (HRT) and substrate concentration on the process performance. The maximal sulfide and nitrite removal rates were achieved to be 13.82 and 16.311 kg/(m³ day), respectively, at 0.10 day HRT. The process can endure high sulfide concentrations, as the sulfide removal percentage always remained higher than 88.97% with influent concentration up to 1920 mg/L. Incomplete sulfide oxidation took place due to lower consumed nitrite to sulfide ratios of 0.93. It also tolerated high nitrite concentration up to 2265.25 mg/L. The potential achieved by decreasing HRT at fixed substrate concentration is higher than that by increasing substrate concentration at fixed HRT. The process can bear short HRT of 0.10 day but careful operation is needed. Nitrite conversion was more sensitive to HRT than sulfide conversion when HRT was decreased from 1.50 to 0.08 day. Stoichiometric analyses and results of batch experiments show that major part of sulfide (89–90%) was reduced by nitrite while some autooxidation (10–11%) was resulted from presence of small quantities of dissolved oxygen in the influent wastewater. There was ammonia amassing in considerably high amounts in the bioreactor when the influent nitrite concentration reached above 2265.25 mg/L. High ammonia concentrations (200–550 mg/L) in the bioreactor contributed towards the overall inhibition of the process. Present biotechnology exhibits practical value with a high potential for simultaneous removal of nitrite and sulfide from concentrated wastewaters at shorter HRT.

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1. Introduction

Biotechnology can be used to assess the well being of ecosystems, transform pollutants into benign substances, generate biodegradable materials from renewable sources, and develop environmentally safe manufacturing and disposal processes. Environmental biotechnology employs the application of microbial metabolism to optimize the efficiency of microorganisms to reduce the environmental burden of toxic substances.

Hydrogen sulfide (H₂S) is toxic to human in addition to imparting severe effects on ecosystems even at very low concentrations. Being the most reduced form of sulfur, sulfide possesses a high oxygen demand of 2-mol O₂/mol S²⁻ causing depletion of oxygen upon discharge of sour wastewater [1]. Various

toxicological effects of sulfide upon human health have been described elsewhere [2–4]. Biogenic production of H₂S in oil and gas reservoirs causes contamination of oil products with sulfur compounds [5]. The activity of sulfidogenic bacteria produces sulfides in the water from secondary oil production [6]. Toxic and corrosive nature of sulfides demands their removal from the produced fluids to prevent emission of sulfur oxides during combustion of fossil fuels [7].

Main sources of nitrogenous species in wastewaters include reject water from wastewater treatment plants (WWTP), excessive use of piggery manure, landfill leachate and industrial wastewaters. Millions of gallons of sodium nitrite rich wastewaters are generated from boiler hydroblasting by the navy operations. This nitrite-rich wastewater is considered as a hazardous waste, costing up to \$3.50 per gallon for its treatment [8]. Nitrites in drinking water can lead to oxygen shortage of newly born children ('blue baby syndrome') and during chlorination of drinking water; carcinogenic nitrosamines may be formed

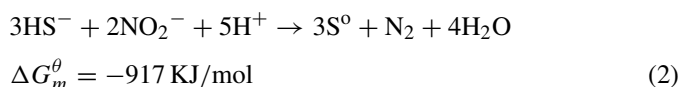
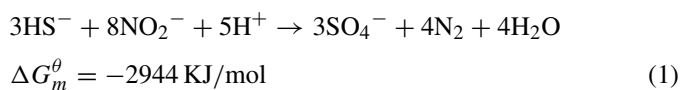
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by the interaction of nitrite with compounds containing organic nitrogen. A wide range of biological and chemical removal systems are available for removal of nitrogenous compounds from wastewaters [9].

Being economic and efficient, biological process has been the most popular technology for wastewater treatment. Biological processes operate in ambient environments without needing expensive chemicals and catalysts. Moreover, biological processes can be used for the treatment of small volumetric flows offering high removal efficiencies (RE) even with low sulfide concentrations [10]. The simpler nutritional and energy requirements has made the chemolithotrophs a favorable candidate for biooxidation of sulfide [11–12].

Some bacterial species like *Thiobacillus denitrificans* can oxidize sulfide to element sulfur simultaneously reducing nitrogenous species to dinitrogen. Despite the significant amount of research on biooxidation of sulfide, the sensitivity of bacteria to high levels of sulfide is the main technical barrier to widespread application of this technology [9]. Because autotrophic denitrifiers utilize inorganic carbon compounds as their carbon source [13], they do not require organic carbon source, resulting in less sludge production and minimizing the excessive sludge disposal cost [14]. Being an oxidized nitrogen form, nitrite can be an effective electron acceptor for sulfide oxidation. The overall biochemical reactions during sulfide oxidation under different sulfide/nitrite molar ratios are shown in (Eq. (1) and (2)), indicating that reactions producing sulfate are thermodynamically more favored.



Increasing attention has focused on the role of nitrate on the oxidation of sulfides by indigenous sulfide-oxidizing bacteria present in oil and gas fields recently [15]. However, surprisingly little attention has been paid to study and optimize simultaneous anoxic sulfide and nitrite removal from waste streams. Considering the limited amount of work on biooxidation of sulfide utilizing nitrite as electron acceptor, further work is clearly required to explore the potential of nitrite based sulfide removal from wastewaters. Being anoxic process, significant aeration cost savings can be realized in ASO reactor for simultaneous removal of sulfide and nitrite. Nitrite being more reactive in aqueous state can serve an efficient electron acceptor for sulfide oxidation.

1.1. Aim of present study

The objective of the present study was to test the potential of anoxic sulfide-oxidizing (ASO) reactor for simultaneous sulfide and nitrite removal from wastewaters through increasing substrate concentration at fixed HRT and decreasing HRTs at fixed substrate concentration.

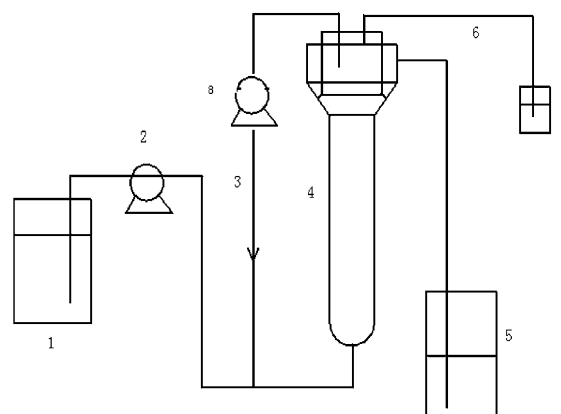


Fig. 1. The schematic presentation of experimental set up. (1) Influent tank, (2) Feeding pump, (3) Recycling port, (4) UASB reactor, (5) Effluent container, (6) Port to gas collector, (7) Gas collector containing water and (8) Recycling pump.

2. Materials and methods

2.1. Anoxic sulfide-oxidizing reactor

The anoxic sulfide-oxidizing reactor is an up flow reactor with biomass retention. 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 was pumped through a peristaltic pump from the 5-L influent vessel to the reactor. The flow rate can vary between 0.6 and 12.5 L per day, which gives the possibility operating at HRTs between 2.0 and 0.1 days. A recycling pump was used in order 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 about 2.5–3.0. The temperature of the reactor can be controlled between 20 and 70 °C using a thermostat, although the normal operational temperature was 30 °C.

2.2. Inoculum and enrichment of microbial communities

Inoculum was taken from the anaerobic methanogenic reactor in Sibao Wastewater Treatment Plant located in Hang Zhou city, China. Its total solids (TS) and volatile solids (VS) were measured as 154.10 and 50.89 g/L, respectively, providing VS/TS ratio of 0.322. ASO reactor was operated under lithoautotrophic conditions where sulfide was used as electron donor and nitrite as electron acceptor to accomplish denitrification. Initially the reactor was inoculated with 0.7 L methanogenic sludge taken from Sibao Waste Water Treatment Plant located in Hang Zhou, China.

2.3. 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). Trace element solution contained $\text{Na}_2\text{-EDTA}$ (50 g/L), NaOH (11 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (11 g/L), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (3.58 g/L), $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$ (2.5 g/L), ZnCl_2 (1.06 g/L), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.5 g/L), $(\text{NH}_4^+)_6$

Mo₇O₂₄·4H₂O (0.5 g/L) and CuCl₂·2H₂O (0.14 g/L). Sodium sulfide (Na₂S·9H₂O) and sodium nitrite (NaNO₂) were added as required. The nitrite–nitrogen and sulfide–sulfur concentrations varied according to the type of experiment. The influent concentrations were 75.75–2265.25 mg N/L and 240–1920 mg S/L. The ratio of sulfide to nitrite was 3:8 while assessing the potential of ASO reactor. The suitable concentrations of sulfide and nitrite during substrate concentration experiment, i.e. 1152.00 and 1359.25 mg/L, respectively, were used during HRT experiment.

2.4. Operational parameters

Operational and performance parameters used for ASO reactor include loading rates, hydraulic retention time (HRT) and removal efficiency. Mass loading rate defines the amount of contaminant entering the ASO reactor per unit volume per unit time. Hydraulic retention time is the time a unit volume of wastewater will remain in ASO reactor and overestimates the actual treatment time. Both terms are normalized, allowing for comparison between reactors of different sizes. Removal efficiency 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 removal efficiency corresponding to maximum microbial substrate utilization rate is observed. This limitation is due to the effect of high concentrations on the Monod kinetics of biodegradation [16]. In some cases, it is known that very high concentration of substrate can become inhibitory [17]. 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. Removal efficiency 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 is measured.

2.5. The start up of ASO reactor and establishment of steady state

Before start up, the reactor was fed with synthetic wastewater in order to enrich the required microbial communities from the inoculum and to acclimatize them to the new substrates. In order to start up, the reactor was set in up flow mode with a HRT of 2.0 days at 30 ± 2 °C in a room having thermostatic device. The concentration of sulfide in the influent was increased from 32.0 to 128.0 mg/L and nitrite from 37.75 to 151.0 mg/L, respectively, keeping HRT at 2 days. Loading rates and removal efficiency were considered as the criteria to reach steady state. Previous investigation achieved the sulfide loading rate of 0.042–0.294 kg/m³ day [18] during start up, while no specific loading for simultaneous removal of sulfide and nitrite was available in literature. Earlier studies achieved only moderate volumetric treatment capacities (<1 g NO₃–N/L day) for combined hydrogen sulfide and autotrophic denitrification [19]. Thus, sulfide loading of 0.3 kg/m³ day with removal efficiency above 90% and nitrite loading of 0.05 kg/m³ day with a removal

efficiency above 50% was regarded as the criteria for successful start up of ASO reactor.

2.6. Batch cultures

Two kinds of batch tests (biotic and abiotic) were carried out in order to determine the nature of reaction between sulfide and nitrite. The assays were carried out in 120 mL serum flasks with butyl rubber stoppers. One hundred milliliters of mineral medium was used with the basal chemical composition listed above. For the biotic test, 12 mL of ASO biomass from ASO reactor was added to each flask for a final concentration of 1.55 g SS/L. The conditions for abiotic tests were similar but no inoculum was added. The pH and temperature were 8.37 and 35 °C, respectively. Sulfide, nitrite and bicarbonate were used at concentrations of 128, 73 and 10 mg/L, respectively.

2.7. Analytical procedures

Ammonium nitrogen (NH₄⁺–N) was measured by phenate method [20], nitrite nitrogen (NO₂[–]–N) was measured by colorimetric method [20] and nitrate nitrogen (NO₃[–]–N) was measured by ultraviolet spectrophotometric screening method [20] on daily basis using spectrophotometer (Unico UV-2102 PC and 722S, China). The sulfide was determined by iodometric method [20] and sulfate was measured by turbidimetric method [20]. The pH measurement was performed according to standard method [20]. A three-point calibration of pH meter was carried out daily. Total solids (TS) concentrations were determined according to gravimetric method at 105 °C (method no. 160.3) and volatile solids were analyzed by gravimetric method at 550 °C [20].

2.8. Statistical work

Statistical calculations were computed by using Microsoft Excel software.

3. Results

3.1. Start up of the ASO reactor

The removal percentage of nitrite and sulfide in the ASO reactor during start up was very high. After the operation of 15 days, the ASO reactor reached steady state as the sulfide and nitrite removal efficiencies were consistently higher than 99 and 75%, respectively (Table 1). After operation for 60 days, the sulfide loading rate (0.30 kg/m³ day) (Table 1) was higher than that reported in the literature, i.e. 0.042–0.294 kg/m³ day [18]. Based on the loading rates, the start up of ASO reactor could be regarded as successful. During start up, the nitrite loading rates were 0.019–0.36 kg/(m³ day).

3.2. Sulfide loading rate

Loading rate is an imperative index to assess the potential of a bioreactor. Two kinds of sulfide loading rates were applied to

Table 1
Steady state performance of the ASO reactor at various loading rates (fixed HRT)

Time (days)	Q, Sulfide-sulfur feed (mg/L)				Q, Nitrite-nitrogen feed (mg/L)				Denitrifying yield		
	Influent	Effluent	Sulfide removal efficiency	Loading rate ^a	Sulfate formed	Influent	Effluent	Nitrite removal efficiency	Loading rate ^a	Nitrogen formed (mg/L)	Y-N ₂ ^b
1	32	0.42 ± 0.12	98.66 ± 0.12	0.02 ± 0.00	499.7 ± 84.91	37.75	5.314 ± 3.89	85.92 ± 3.89	0.019 ± 0.00	16.99 ± 5.78	0.45 ± 0.15
15	128	0.80 ± 0.15	99.38 ± 0.15	0.06 ± 0.00	573 ± 30.92	151	32.10 ± 2.69	78.75 ± 2.69	0.076 ± 0.00	117.22 ± 4.54	0.77 ± 0.03
45	448	0.55 ± 0.05	99.88 ± 0.05	0.22 ± 0.00	212 ± 57.81	528.75	134.67 ± 25.12	74.52 ± 25.12	0.264 ± 0.01	393.8 ± 25.5	0.74 ± 0.05
60	608	2.73 ± 2.19	99.71 ± 2.19	0.30 ± 0.00	404 ± 50.98	717.75	153.36 ± 25.20	78.62 ± 25.20	0.359 ± 0.01	559.47 ± 17.67	0.77 ± 0.02
75	768	0.69 ± 0.51	99.90 ± 0.51	0.38 ± 0.00	310 ± 27.45	906.25	190.81 ± 18.45	78.94 ± 18.45	0.453 ± 0.01	700.87 ± 15.86	0.77 ± 0.02
90	1024	1.02 ± 0.17	99.90 ± 0.17	0.51 ± 0.00	348 ± 59.20	1208.25	266.56 ± 30.75	77.93 ± 30.75	0.604 ± 0.02	929.45 ± 43.05	0.76 ± 0.04
105	1408	1.63 ± 0.29	99.87 ± 0.29	0.70 ± 0.00	355 ± 44.48	1661.25	327.1 ± 2.78	72.39 ± 2.78	0.831 ± 0.00	1334.15 ± 2.78	0.80 ± 0.00
120	1664	1.42 ± 0.29	99.9 ± 0.29	0.83 ± 0.00	224 ± 27.10	1963.25	412.83 ± 53.67	78.96 ± 53.67	0.982 ± 0.03	1550.41 ± 53.67	0.78 ± 0.03
135	1920	211.77 ± 77.8	88.97 ± 77.8	0.96 ± 0.00	Zero	2265.25	1122.06 ± 49.75	50.46 ± 49.75	1.13 ± 0.02	619.50 ± 52.92	0.27 ± 0.02

^a (kg/(m³ day)).
^b (mgN₂/mg NO₂-N).

assess the potential of ASO reactor after start up. Initially, the influent sulfide concentration was increased up to 1920 mg/L at regular increments at fixed HRT of 2 days (Table 1). The sulfide loading rates ranged 0.02–0.96 kg/(m³ day) and the sulfide removal percentage was higher than 88.97%. The sulfide in the effluent was lower than 2 mg/L until 120 days of reactor operation. During final 15 days of operation, there was a gradual decrease in sulfide removal percentage accompanied by a rise in the effluent sulfide concentrations that elevated to 211 mg/L (Table 1). The reactor operation terminated after 135 days due to substrate toxicity caused by very high influent nitrite and sulfide, i.e. 2265.25 and 1920 mg/L, respectively.

In second instance the influent sulfide concentration was kept unchanged at 1152 mg/L (optimal substrate concentration during substrate concentration experiment) simultaneously decreasing HRT from 1.5 to 0.08 days (Table 2A). During HRT experiment, the sulfide loading rate ranged from 0.77 up to 13.82 kg/(m³ day), and the removal efficiency remained higher than 99.16% until 0.1 day HRT. The sulfide removal rate declined sharply to 96.66% at 0.08 days HRT due to substrate toxicity (Table 2A).

3.3. Nitrite loading rate

Two kinds of loading tests were conducted for nitrite as described for sulfide. At fixed HRT of 2 days, the influent nitrite concentration was increased incrementally from 37.75 to 2265.25 mg/L (Table 1). The loading rate ranged from 0.02 to 1.13 kg/(m³ day) and the removal percentage was in the range of 85.92–50.46% (Table 1). At decreasing HRT (1.5–0.08 day), the influent nitrite concentration was kept unchanged at 1359.25 mg/L, the nitrite loading rate was in the range of 0.91–16.31 kg/(m³ day), while the removal percentage ranged from 85.50 to 54.56% (Table 2B).

3.4. Effect of HRT

Throughout the experiment, HRT had little impact on sulfide removal percentage. When decreasing HRT from 1.5 to 0.10 days, the effluent sulfide concentration remained lower than 2 mg/L, and removal percentage always maintained higher than 99.16% (Table 2A). The process deteriorated abruptly as the HRT was further decreased to 0.08 day causing decrease in the sulfide removal efficiency to 96.66%. Inversely HRT had a notable sway on nitrite removal percentage in the tested range. At decreasing HRTs, nitrite removal percentage always remained around 80% until 0.1 day. However, as HRT was decreased from 0.10 to 0.08 days, effluent nitrite concentration elevated to 617.56 mg/L and removal percentage dropped to 54.56% (Table 2B).

3.5. Ammonia accumulation

Ammonia exhibited a very interesting behavior during the experiment. Whenever influent nitrite and sulfide were increased there was meager ammonia appearance, which was not high enough to hamper the function of ASO reactor. The ammo-

Table 2A
Average performance of the ASO reactor at decreasing HRTs

HRT (days)	Effluent sulfide concentration (mg/L)	Sulfide loading rate (kg/(m ³ day))	Sulfide removal efficiency	Sulfate formed (mg/L)
1.50	2.06 ± 0.39	0.768 ± 0.0	99.80 ± 0.04	333.32 ± 31.5
1.25	1.98 ± 0.49	0.92 ± 0.0	99.82 ± 0.04	352.94 ± 5.25
1.00	1.61 ± 0.86	1.15 ± 0.0	99.85 ± 0.08	299.23 ± 43.29
0.87	1.67 ± 0.55	1.31 ± 0.0	99.85 ± 0.04	291.46 ± 15.48
0.75	1.49 ± 0.42	1.53 ± 0.0	99.86 ± 0.04	234.45 ± 9.54
0.62	1.40 ± 0.34	1.84 ± 0.0	99.87 ± 0.03	277.42 ± 39.75
0.50	2.29 ± 0.76	2.30 ± 0.0	99.79 ± 0.06	253.52 ± 25.01
0.37	3.25 ± 0.11	3.07 ± 0.0	99.71 ± 0.00	221.96 ± 16.94
0.25	5.21 ± 1.11	4.60 ± 0.0	99.54 ± 0.10	190.17 ± 4.46
0.16	6.77 ± 0.31	6.91 ± 0.0	99.41 ± 0.02	169.47 ± 18.28
0.12	7.62 ± 0.23	9.21 ± 0.0	99.33 ± 0.02	196.92 ± 1.06
0.10	9.63 ± 0.06	11.05 ± 0.0	99.16 ± 0.01	179.31 ± 20.49
0.08	38.42 ± 0.21	13.82 ± 0.0	96.66 ± 0.03	6.67 ± 0

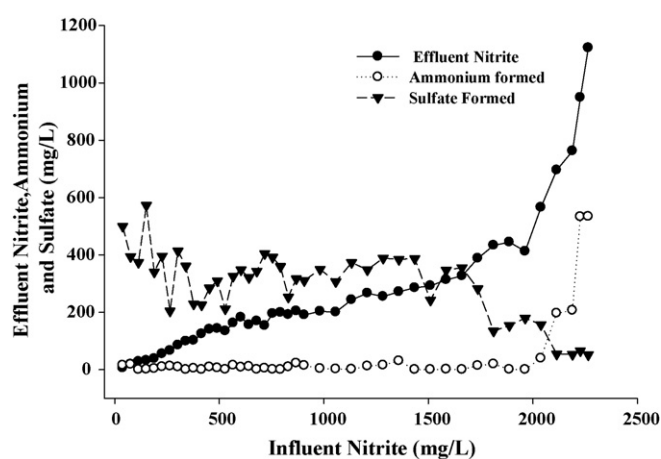


Fig. 2. Relation between influent and effluent nitrite, ammonium and sulfate during loading test at fixed HRT.

nia concentration was considerably elevated when the influent nitrite concentration was above 2000 mg/L with fixed HRT of 2 days (Fig. 2). This ammonia amassing was correlated to high effluent nitrite concentration during the final stages of experiment. Same behavior was ubiquitous while studying the effect of

HRTs on the process performance; this effect has been depicted in Table 2B. The relation between the influent nitrite and ammonium accumulation during loading tests has been presented in Fig. 2. It is very attention-grabbing that ammonia accumulation was always accompanied by elevated levels of effluent nitrite in the ASO reactor.

3.6. Batch cultures

A series of batch culture experiments were conducted to find out whether the reaction is biological or merely chemical. The Gibbs free energy changes (ΔG^{θ}) associated with the oxidation of sulfide in the presence of nitrite are exergonic (-2944 kJ/mol) (Eq. (1)). So the reaction should occur spontaneously under standard conditions. Moreover, the reaction producing sulfate is thermodynamically more favored (Eq. (1) and (2)). Experiments conducted under lithotrophic conditions showed that biological sulfide removal was complete while nitrite conversion efficiency was close to 90%. Under abiotic conditions, 11% of sulfide disappeared and 6% of NO_2^- was reduced to N_2 . Thus, because the biological conversions were far higher than the abiotic ones, the denitrification process was predominant.

Table 2B
Average performance of the ASO reactor at decreasing HRTs

HRT (days)	Effluent concentration (mg/L)	Nitrite loading rate (kg/(m ³ day))	Nitrite removal percentage/(%)	Effluent ammonium concentration (mg/L)
1.50	245.51 ± 4.35	0.906 ± 0.0	81.93 ± 0.31	Zero
1.25	264.88 ± 7.11	1.087 ± 0.0	80.51 ± 0.52	Zero
1.00	196.74 ± 62.40	1.360 ± 0.0	85.50 ± 4.59	Zero
0.87	197.43 ± 3.53	1.553 ± 0.0	85.47 ± 0.26	Zero
0.75	232.17 ± 2.49	1.812 ± 0.0	82.91 ± 0.18	Zero
0.62	267.91 ± 3.09	2.175 ± 0.0	80.289 ± 0.22	Zero
0.50	271.14 ± 5.63	2.719 ± 0.0	80.04 ± 0.41	32.34 ± 3.80
0.37	271.92 ± 2.39	3.625 ± 0.0	80.00 ± 0.17	36.18 ± 9.59
0.25	276.47 ± 9.08	5.437 ± 0.0	79.66 ± 0.66	32.99 ± 1.55
0.16	282.42 ± 3.76	8.156 ± 0.0	79.22 ± 0.27	56.79 ± 22.13
0.12	291.94 ± 4.42	10.874 ± 0.0	78.52 ± 0.32	63.62 ± 2.19
0.10	276.94 ± 5.74	13.047 ± 0.0	79.84 ± 0.42	114.18 ± 56.78
0.08	617.56 ± 0.819	16.311 ± 0.0	54.56 ± 0.19	375.85 ± 1.90

4. Discussions

There has been a considerable amount of interest in the past 10–15 years in the design of nitrification–denitrification systems employing nitrite as the primary intermediate – thereby eliminating the formation of nitrate – in both the nitrification and denitrification steps. Numerous authors [21–26] have reported the capital and operational benefits of the process, referred to here as the nitrate shunt; to include a 25% reduction in aeration requirements, a 40% reduction in external carbon addition for denitrification, a potential reduction in anoxic zone volume, and a significant reduction in sludge production. Being anoxic process, significant aeration cost savings is realized in ASO reactor for simultaneous removal of sulfide and nitrite.

Very high sulfide loading rates (i.e. 13.82 kg/m³ day) were achieved for present investigation which can be compared with that obtained by earlier studies utilizing chemotrophic and phototrophic systems for treatment of synthetic effluents from H₂S scrubber, anaerobic effluents and effluents of paper mills [1,27–33]. Present investigation achieved one of highest loading rates as obtained by previous work of Janssen et al. (14 kg/m³ day) [33] who treated anaerobic effluents using sulfur sludge. Previous investigations achieved sulfide removal rates in the range of 70–99% [27–33], while the maximum sulfide and nitrite removal percentages for present study were 99.90 and 85.92%, respectively. Likewise, the influent sulfide concentration used previously were in the range of 1.0–294 mg/L [27–33] which are much lower than present work with a maximum sulfide concentration of 1920 mg/L. Thus, our influent sulfide concentration (1920 mg/L) is the highest concentration applied for simultaneous removal of nitrite and sulfide. Upon the increase in the influent sulfide concentration up to 1920 mg/L, the effluent sulfide concentration elevated to 211.77 mg/L with a drop in the sulfide removal percentage to 88.97%. It implies that very high influent concentrations of 1920 mg/L caused a severe inhibition to the microbial activity. Judged by the sulfide conversion, the optimum sulfide concentration can possibly be 1728 mg/L.

It is clear from results that ASO reactor displayed very strong tolerance to high sulfide and nitrite concentrations of 1920.0 and 2265.25 mg/L, respectively. In addition it tolerated a very short HRT of 0.08 day. These findings are of practical value as very concentrated wastewaters can be treated in ASO reactor at a very short HRT for simultaneous removal of sulfide and nitrite saving operational cost.

Being a reduced form sulfide can be oxidized into sulfate or elemental sulfur depending upon the availability of electron acceptor. Batch culture experiments and stoichiometric analysis was performed to underpin the nature of biological reaction. According to Eqs. (1) and (2), the theoretical nitrite to sulfide ratio is 1.17 ($8 \times 14/3 \times 32$) if sulfide is oxidized to sulfate and the ratio is 0.29 ($2 \times 14/3 \times 32$) if sulfide is oxidized to sulfur. Observed ratio of nitrite to sulfide at various loading rates was 0.93, which is closer to theoretical ratio for oxidization of sulfide to sulfate than that for oxidization of sulfide to sulfur. Judged by the ratio of sulfate produced to sulfide removed (data calculated

from Table 1), we could see that the reaction of oxidization of sulfide to elemental sulfur predominates in the reactor.

Nitrite reduction and its contribution to sulfate formation were also analyzed. According to Eq. (1) we would expect to have 3/8 mol produced sulfates per mole of nitrite or 0.78 mg SO₄ produced/mg NO₂ consumed. In Table 1 for example at 528.75 mg influent nitrite we find 134.67 mg/L nitrite in the effluent. This means that 394.08 mg of nitrite were consumed so we would expect to have $394.08 \times 0.78 = 307.38$ mg/L of sulfate, but we have only around 212 mg/L. Based on discussion above it can be concluded that some sulfide must have consumed in elemental sulfur formation. Likewise, sulfide oxidation at 1661.25 mg/L nitrite can be analyzed for sulfate formation. At this point the effluent nitrite was 327.1 mg/L, which implies that 1334.15 mg/L nitrite contributed to sulfide oxidation. The expected sulfate formation for 1334.15 mg/L nitrite should be $1334.15 \times 0.78 = 1040.63$ mg/L. However, just 355 mg/L sulfate was recorded at this nitrite concentration indicating incomplete sulfide oxidation. Some unusual amounts of sulfate formed on days 1 and 15 (499 ± 84.91 and 573 ± 30.92 , respectively) were noted at influent sulfide levels of 32 and 128 mg/L, respectively. This might be due to some faulty sulfate determination as theoretically and stoichiometrically it is impossible to form that much sulfate. The results from batch experiments revealed that 11% sulfide disappeared under abiotic conditions. Sulfide is readily autooxidizable at normal temperatures, which is as fast as biological reaction [34]. It is logical to conclude that about 10–11% of sulfide might have been autooxidized due to presence of some dissolved oxygen in inlet wastewater. Thus, major portion of sulfide oxidation (89–90%) might have occurred by reducing nitrite, while its rest was autooxidized by small quantities of dissolved oxygen in wastewater.

To get an insight into the mechanism of respiratory process, the reduction of nitrite was analyzed at each loading rate. Stoichiometry of Eq. (1) suggests that each mg of nitrite reduced produces 0.6 mg of dinitrogen. The results indicated that at 1963 mg/L nitrite and 1664 mg/L sulfide, 78.96% of nitrite was reduced to dinitrogen giving a denitrifying yield of 0.78 ± 0.03 mgN₂/mg NO₂-N (Table 1) that corresponds to the stoichiometric values. Further increase in the inlet nitrite resulted in a gradual decrease in denitrifying efficiency to 50.46% with 0.27 mgN₂/mg NO₂-N values for Y-N₂. This drop of denitrifying efficiency might be attributed to substrate inhibition caused by very high inlet sulfide and nitrite concentrations accompanied by dissimilatory reduction of nitrite to ammonia. Nitrite is toxic to microbial processes and cellular growth [35]. Nitrite concentration of 390 mg/L inhibited 50% of the activity of *Pseudomonas* cultures [35]. The nitrite consumption agreed with the stoichiometric values of Eq. (1).

In general, higher substrate concentrations will improve treatment efficiency up to a point where biological kinetics drives the degradation rate to zero order. At fixed HRT toxic substrate increments did not inhibit the substrate utilization. The concentration of sulfide and nitrite 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 [36]. However, when maximum rate is reached beyond

which substrate concentrations become inhibitory, causes a decrease in reaction rate. Such kind of inhibition is called as self-inhibition, which is also called as Haldane or Andrews's kinetics [36]. Decreasing of removal efficiency was observed with a lag period after increasing the inlet concentration with subsequent increase in the removal efficiency after gradual acclimation of microbes to the pollutant. At fixed substrate concentration with decreasing HRTs, the reactor can tolerate shorter HRTs with high influent loading rates if specific growth rate of microorganisms under the conditions is greater than the washout rate (the inverse of HRT). However, if the influents are highly concentrated at a fixed HRT, the specific growth rate of bacteria can be inhibited resulting in the build up of toxic substrates in the reactor.

The formation of ammonia was accompanied by higher effluent nitrite, and might be inhibitory to nitrite reducers present in the reactor (Fig. 2). To date, free ammonia is the consensus cause of nitrite oxidizer inhibition. Nitrate or nitrite can be reduced to a gas species or ammonia. If nitrate or nitrite is reduced to ammonia, it can accept more electrons and it occurs more frequently when acceptor is in short. Surmacz-Gorska et al. [37] reported that $\text{NH}_4\text{-N}$ concentration of 500 mg/L caused nitrite accumulation up to 300 mg N/L in activated sludge at pH 8.0. In another case $\text{NH}_4\text{-N}$ concentration up to 1060 mg/L had no effect on nitrite accumulation in enriched culture at pH range of 6.6–8.0 [38].

5. Conclusions

The main features of present investigation are as follows:

- (1) ASO reactor can treat high influent concentrations of sulfide (1920 mg/L) and nitrite (2265.25 mg/L) effectively. These influent concentrations are one of the highest influent concentrations applied during anoxic treatment of sulfide-rich wastewaters. The maximum loading rates for sulfide and nitrite, i.e. 13.84 kg S/(m³ day) and 16.311 kg N/(m³ day), respectively, at 0.10 days HRT were obtained. Stoichiometric analysis suggested that because of lower consumed sulfide to nitrite ratios of 0.93, incomplete sulfide oxidation took place resulting in sulfur as major oxidation product. Stoichiometric analyses and results of batch experiments show that major part of sulfide (89–90%) was reduced by nitrite while some autooxidation (10–11%) resulted from presence of small quantities of dissolved oxygen in the influent wastewater.
- (2) HRT had little impact on sulfide removal percentage while it exerted a notable sway on nitrite removal percentage for the tested range. The process can tolerate lower HRT of 0.10 day but careful operation is needed while decreasing HRT. Nitrite conversion is more sensitive to HRT than sulfide conversion since the process performance deteriorated abruptly when HRT was decreased from 0.10 to 0.08 days. High influent concentrations and short HRT achieved by present study implies that ASO reactor can treat concentrated wastewaters containing sulfide and nitrite simultaneously within a short period of time saving operational cost.
- (3) Considerably higher amounts of ammonia accumulated in bioreactor when the influent nitrite concentration was above 2000 mg N/L with fixed HRT at 2 days. Ammonia accumulation coincided with higher effluent nitrite during final stages of the experiment. Same behavior was prevalent while studying the effect of HRTs on the process performance. High ammonia concentrations in the bioreactor contributed towards the overall inhibition of the process.

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