Nitrite and nitrate as electron acceptors for biological sulphide oxidation

G. Munz, A. Mannucci, J. Arreola-Vargas, F. Alatriste-Mondragon, F. Giaccherini and G. Mori

ABSTRACT

Autotrophic denitrification with sulphide using nitrate (R1) and nitrite (R2) as electron acceptor was investigated at bench scale. Different solids retention times (SRT) (5 and 20 d) have been tested in R1 while R2 was operated at SRT = 13 d. The results indicated that the process allows complete sulphide removal to be achieved in all tested conditions. Tested sulphide loads were estimated from the H_2S produced in a pilot-scale anaerobic digester treating vegetable tannery primary sludge; nitrogen loads originated from the nitrification of the supernatant. Average nitrogen removal efficiencies higher than 80% were observed in all the tested conditions once steady state was reached. A maximum specific nitrate removal rate equal to 0.35 g $N\text{-}NO_3^-$ g VSS^{-1} d^{-1} was reached in R1. Due to sulphide limitation, incomplete denitrification was observed and nitrite and thiosulphate tend to accumulate especially in the presence of variable environmental conditions in both R1 and R2. Lower SRT caused higher $NO_2^{accumulated}/NO_3^{reduced}$ ratios (0.22 and 0.24, with SRT of 5 d and 20 d, respectively) using nitrate as electron acceptor in steady-state condition. Temperature decrease caused sudden $NO_2^{accumulated}/NO_3^{reduced}$ ratio increase in R1 and NO_2^- removal decrease in R2.

Key words | anoxic processes, denitrification, denitritation, nitrate, nitrite, sulphide biological oxidation

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INTRODUCTION

Sulphide management represents a major problem in many waste water treatment plants (WWTPs) and specific industrial areas as the Tuscan tannery district (Italy), the second largest in Europe, in which more than 5,300,000 m^3 per year of tannery wastewater are produced. In this context, Cuoiodepur WWTP (San Miniato, Pisa, Italy) treats almost exclusively vegetable tannery wastewaters characterized by high chemical oxygen demand (COD) concentration (12–23 $gO_2 L^{-1}$), suspended solids (6–31 $gSS L^{-1}$), ammonium (0.12–0.25 $gN\text{-}NH_4^+ L^{-1}$), chlorides (0.3–8 $g L^{-1}$) and sulphate (1.7–2.7 $gSO_4^{2-} L^{-1}$) (Mannucci et al. 2010), and produces fertilizers from sludge after thermal drying and mixing with by-products of industrial tannery process.

Since European regulation on land application is becoming increasingly stringent, it is important to evaluate alternative options such as anaerobic digestion of primary sludge (Dhayalan et al. 2007; Zupančič & Jemec 2010; Sri Bala Kameswari et al. 2012).

In the last 2 years, an average of 1,200 $m^3 d^{-1}$ of sludge has been wasted from the primary settler of the Cuoiodepur

WWTP and sent to a thickener to reduce water content and double the solids concentration. A future implementation of the anaerobic digestion phase to treat all the thickened primary sludge will require a 15,000 m^3 digester operated with an SRT of 25 d.

However, the presence of tannins is critical for methanogenic bacteria and may affect the outcome of the competition between sulphate-reducing bacteria (SOB) and methanogens (Mannucci et al. 2014).

The SOB activity causes high sulphide concentration in the produced biogas and makes sulphide removal mandatory before methane utilization.

Up until today, chemical scrubbing is the most established technology for H_2S removal in WWTPs (Gabriel et al. 2004) due to several advantages: short contact time (1.3–2 seconds), low capital cost, easy operation and the possibility of treating highly variable loads. However, chemical scrubbing requires large amounts of chemicals, primarily NaOH, that increase both the operating costs and carbon footprint of the treatment. Biological

processes, based on the use of aerobic biotrickling filters (BTFs), do not require the use of chemicals, can remove H_2S under varying operating conditions and require only a few days for biological process start-up (Wu *et al.* 2001; Namini *et al.* 2008).

The use of alternative electron acceptors, instead of oxygen, would make the use of BTF technology more favourable for the treatment of the biogas produced from anaerobic digestion of tannery industrial sludge. The supernatant originated from the anaerobic digestion is characterized by high ammonia concentration (up to 900 mg $N-NH_4^+$ L^{-1}); its nitrification in a side stream separated from the main treatment train will allow the production of liquid streams with high nitrate and/or nitrite concentration that could be used as electron acceptor in autotrophic denitrification process.

Although autotrophic denitrification with sulphide as electron donor has been investigated by several researchers with the main applicative purpose of applying it in the treatment of liquid streams (Lu *et al.* 2009), sulphide removal through denitrification was recently tested on biogas and other gaseous streams (Kleerebezem & Mendez 2002).

To achieve the primary objective of hydrogen sulphide removal, the influent ratio between sulphur and nitrogen has to be considered; nonetheless, it is important to highlight that the stoichiometry of the process is flexible, since sulphide can be oxidized to either elemental sulphur, thiosulphate or sulphate, and nitrogen can be used as either nitrite or nitrate, with the result of significantly widening the range of potentially suitable S/N ratio values. Moreover, the successful application of a BTF for biogas treatment depends on the possibility of controlling biomass growth (Mannucci *et al.* 2012). Both of the above-mentioned issues definitely depend on the bioprocesses' stoichiometry and kinetics, which are, to the best of our knowledge, not clearly quantified in all the reactions of interest. For instance, most previous work on denitrification with sulphide focussed on nitrate as electron acceptor, while the use of nitrite as electron acceptor, despite some exceptions (Moraes *et al.* 2012), is not well investigated and needs to be deepened. Moreover, most previous work on the kinetics and stoichiometry of sulphur compound oxidation was carried out with thiosulphate as electron donor (Artiga *et al.* 2005), while very few tests (Munz *et al.* 2009) have been carried out with sulphide due to the difficulty of using a strongly volatile and, at the same time, inhibiting substrate. This work is aimed at investigating denitrification and denitritation with sulphide, in the presence of a typical S/N ratio that can derive from the anaerobic digestion of primary sludge of tannery wastewater.

MATERIALS AND METHODS

The present experimentation has been conducted at CER^2CO Laboratory, located at the Cuoiodepur WWTP. Two identical bench-scale sequencing batch reactors (SBRs) (R1 and R2) with working volume of 3.2 L and HRT of 24 h, were used (Figure 1). Influent nitrogen and sulphide loads were chosen on the basis of the results of an experimentat (data not shown) conducted using a pilot-scale anaerobic digester (volume = 150 L, SRT = 25 d) fed with sludge from Cuoiodepur WWTP primary settler. The $S-SO_4^{2-}$ concentration in the primary sludge has been monitored daily for more than 250 days (from 28th January to 17th November 2013). The average sulphate concentration and the $S-SO_4^{2-}/S_{tot}$ were 740 ± 200 mg L^{-1} $S-SO_4^{2-}$ and 0.96, respectively. Anaerobic digester effluent $S-SO_4^{2-}$ and effluent $S-S^{2-}$ were 15 mg S L^{-1} and 13 mg S L^{-1}, respectively. Experimental data confirmed that 96% of the influent sulphur (as sulphate) was reduced to H_2S that exits the digester as the biogas. Ammonia concentration in the supernatant resulted 722 ± 60 mg N L^{-1}.

An average S/N ratio for the autotrophic denitrification of 1.02 gS gN^{-1} has been calculated.

An S/N ratio of 1.4 constitutes the worst estimated conditions (maximum sulphide and minimum nitrate/nitrite) for the main objective of complete sulphide removal. S/N ratios similar to those obtained during anaerobic digestion experimentation were tested as influent conditions in both SBR reactors.

R1 was fed filled with 1 L (total suspended solids (TSS) = 8,000 mg L^{-1}) of activated sludge collected from Cuoiodepur WWTP biological section and diluted with tap water. The reactor was fed with two distinct peristaltic pumps with sulphide (Solution A) and with a solution of micronutrients and either nitrate or nitrite (Solution B and C, respectively). To obtain sludge capable of autotrophic denitrification, R1 activated sludge was acclimatized by feeding synthetic wastewater (Solution A and Solution B) under anoxic condition for more than 40 days.

R1 was operated for 160 days, while R1 biomass was used as inoculum for R2 after 100 days. R1 experimentation was divided into three phases with different operational conditions, as reported in Table 1.

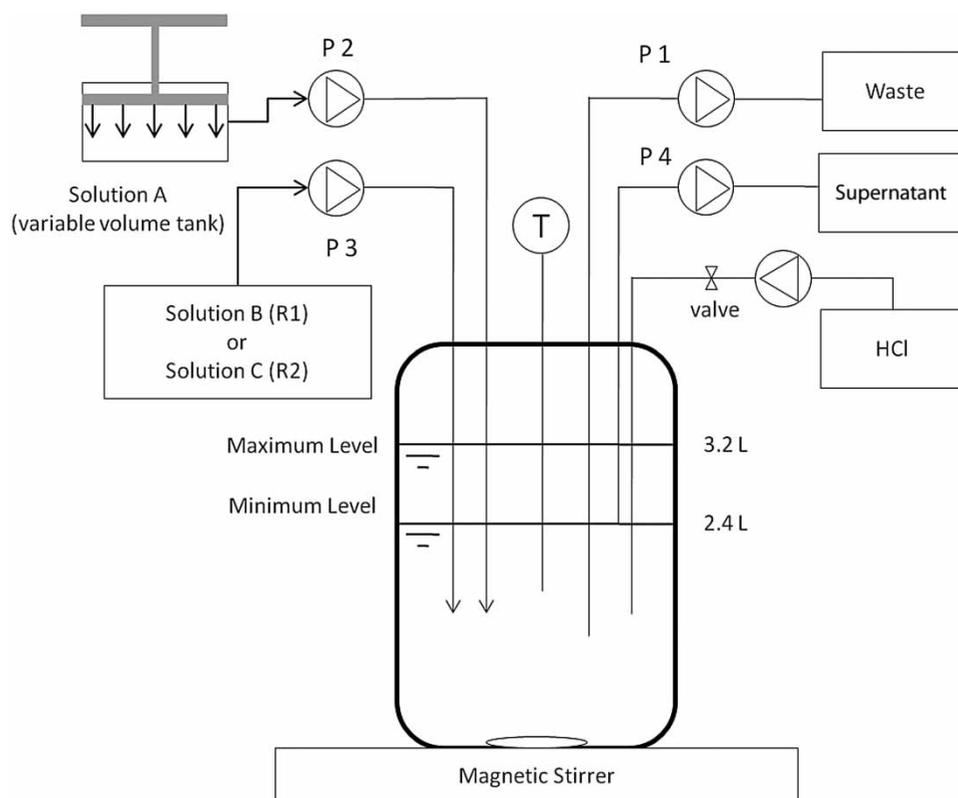Solution A was stored in the absence of headspace, that is, in a storage tank with a variable volume, and at pH = 10

Figure 1 | Schematic of the used bench-scale SBR.

Table 1 | Operational conditions in R1 and R2

Phase		Start-up	I	II	III	III
Parameter	Units	R1				R2
Duration	d	0–24	25–45	46–91	92–160	100–160
S_{IN}	mg S-HS$^-$ d^{-1}	34–140	340	550	550	342
N_{IN}	mg N-NO$_3^-$ d^{-1}	65–125	250	350	350	–
	mg N- NO$_2^-$ d^{-1}	–	–	–	–	320
SRT	d	–*	–*	20	5	13
S/N	g S (g N)$^{-1}$	0.52–1.36	1.36	1.57	1.57	1.05

*Not controlled.

to minimize the desorption of hydrogen sulphide. For Solution A, NaHCO$_3$ (1.24 g L^{-1}) and Na$_2$S·3H$_2$O (0.41 g L^{-1}) diluted in demineralized water were used; for Solutions B and C, Na$_2$HPO$_4$·2H$_2$O (0.66 g L^{-1}); KH$_2$PO$_4$ (0.52 g L^{-1}); NH$_4$Cl (0.05 g L^{-1}); MgSO$_4$ · 7H$_2$O (0.063 g L^{-1}); KNO$_3$ (1.63 g L^{-1}; B only) and NaNO$_2$ (0.96 g L^{-1} C only) were diluted in tap water.

The cycle phases for both reactors were as follows: feeding (30 min); mixing (60 min); settling (240 min); decant (30 min). The excess biomass was removed during

the last 2 min of the mixing phase. The reactors were maintained at pH between 7 and 8 through the dosage of an HCl solution and at room temperature (between 18 and 28 °C).

Samples were collected three times a week and the following parameters were monitored: COD (soluble and total), volatile suspended solids (VSS), TSS, N-NO$_2^-$, N-NO$_3^-$, S-SO$_4^{2-}$, sulphide, total sulphur, temperature (T), pH. Thiosulphate and elemental sulphur were estimated indirectly. Soluble and total COD, TSS and VSS

were analysed according to IRSA-CNR methods (Metodi analitici per le acque 2003). $S\text{-}SO_4^{2-}$, sulphide, nitrite and nitrate were measured through ionic chromatography (ICS1000, Dionex, Sunnyvale, USA) while total sulphur was measured using plasma spectrophotometry (ICP-OES, Agilent Technology, Santa Clara, USA). A portable Hach-Lange (Berlin, Germany) probe was used to measure the pH twice a day in the effluent. Elemental sulphur and thiosulphate were estimated from the results of total and soluble COD in the wasted mixed liquor. Thiosulphate remains in soluble form and contributes to the soluble COD, while elemental sulphur remains in particulate and colloidal form, and its contribution to the total COD have been estimated on the basis of the difference between total and soluble COD. Thiosulphate and elemental sulphur concentration in the reactors was estimated according to Equations (1) and (2):

$$S_{\text{thiosulphate}} = \left(COD_S - N_{\text{nitrite}} \frac{gCOD}{gN_{\text{nitrite}}} \frac{gCOD}{gS_{\text{thiosulphate}}} \right) \frac{MW_{\text{sulphur}}}{MW_{\text{thiosulphate}}} \quad (1)$$

$$S_{\text{elemental sulphur}} = \left(COD_P - \frac{VSS}{TSS} \frac{gCOD}{gVSS} TSS \right) \frac{gCOD}{gS_{\text{elemental sulphur}}} \quad (2)$$

where MW = molecular weight.

RESULTS AND DISCUSSION

R1 performance evaluation

During the whole experimentation, sulphide removal efficiency (RE) in R1 was always higher than 99% (data not shown). These results are the same as those reported by Fajardo et al. (2012) in SBR reactors treating sulphide and nitrate in sulphide-limiting conditions.

However, a sulphur mass balance based on a complete sulphide oxidation into sulphate does not explain the observed RE. The loss of sulphur estimated from the difference between theoretical sulphate production from a complete sulphide oxidation and experimental sulphate production was 17% and 18% during phase II and phase III, respectively. The analysis of R1 effluent in steady-state conditions confirms the presence of intermediate sulphur compounds as thiosulphate and elemental sulphur (Table 2).

Nitrate specific denitrification rate (SDR) and nitrite accumulation rate indicate an incomplete denitrification (Figure 2).

An incomplete denitrification was observed during the whole experimentation. After nitrogen load increase at day 25 (from 80 to 160 mg $N\text{-}NO_3^-$ L^{-1}), nitrate SDR increased from 0.025 to 0.1 g $(N\text{-}NO_3^-)$ g $(VSS)^{-1}$ d^{-1}. At the same time, nitrite started to accumulate in R1. During phase III with an SRT of 5 d, nitrate SDR doubled and reached a maximum value of 0.35 g $(N\text{-}NO^{3-})$ g $(VSS)^{-1}$ d^{-1}. These values

Table 2 | Sulphur and nitrogen mass balance in R1 and R2 in steady-state conditions and characterization of influent and effluent compounds

Reactor	Parameter	IN (mg d^{-1})	S$_{tot,IN}$ (mg d^{-1})	N$_{tot,IN}$ (mg d^{-1})	OUT (mg d^{-1})	S$_{tot,OUT}$ (mg d^{-1})	S-Diff. (%)	N-RE (%)
R1 Phase II	S-SO$_4^{2-}$	109 ± 20	662	–	552 ± 49	645	2.6	–
	S-S^{2-}	552 ± 32			0			
	S-S$_2$O$_3^{2-}$	0			92.5			
	S-S$_0$	0			0			
	N-NO$_2^-$	0	–	350	53.5	96.5	–	74
	N-NO$_3^-$	350 ± 10			43			
R1 Phase III	S-SO$_4^{2-}$	105 ± 18	695	–	576 ± 80	647	6.9	–
	S-S^{2-}	589 ± 54			0			
	S-S$_2$O$_3^{2-}$	0			64.6			
	S-S$_0$	0			6.4			
	N-NO$_2^-$	0	–	350	39.8	70.3	–	80
	N-NO$_3^-$	350 ± 15			30.5			
R2	S-SO$_4^{2-}$	101 ± 10	434	–	405 ± 24	477	9.1	–
	S-S^{2-}	332 ± 21			0			
	S-S$_2$O$_3^{2-}$	0			72 ± 8			
	S-S$_0$	0			0			
	N-NO$_2^-$	320 ± 15	–	320	75.2	75.2	–	76.5

Difference between influent and effluent total sulphur is reported as S-Diff (%) while nitrogen RE is reported as N-RE.
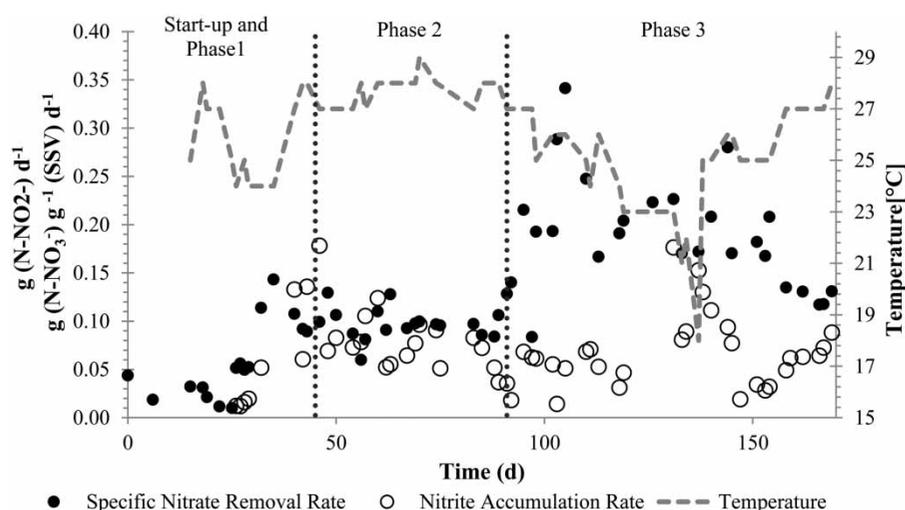
Figure 2 | Nitrate SDR, nitrite accumulation rate and temperature trend in R1 experimentation.

are similar to those obtained by Fajardo *et al.* (2012) in similar conditions. Nitrate RE varied from 67 to 93% during phase II and III, and no clear effects of temperature variations were registered.

Starting from day 24, a minimum nitrite concentration of 4 mg $N\text{-}NO_2^-\,L^{-1}$ was registered during the experimentation. Nitrite accumulation rate increased from $0.05\,g\,(N\text{-}NO_2^-)\,d^{-1}$ to $0.17\,g\,(N\text{-}NO_2^-)\,d^{-1}$ at day 130.

By day 130, nitrite and thiosulphate increased from 13 mg $N\text{-}NO_2^-\,L^{-1}$ to 73 mg $N\text{-}NO_2^-\,L^{-1}$ and from 5 mg $S\text{-}S_2O_3^-\,L^{-1}$ to 25 mg $S\text{-}S_2O_3^-\,L^{-1}$, respectively. Nitrite and thiosulphate remained higher than 22 mg $N\text{-}NO_2^-\,L^{-1}$ and 8 mg $S\text{-}S_2O_3^-$ L^{-1} until day 142. At day 144, thiosulphate was 6.5 mg $S\text{-}S_2O_3^-\,L^{-1}$ and remained stable until the end of the experimentation. In the same period nitrite concentration was stable at 5 mg $N\text{-}NO_2^-\,L^{-1}$.

An average $NO_2^{accumulated}/NO_3^{reduced}$ ratio of 0.23 was observed. This value is close to the ratio obtained by Fajardo *et al.* (2012) under sulphide-limiting conditions.

Nitrite accumulation might be explained by both sulphide limitations (Manconi *et al.* 2007; Fajardo *et al.* 2012) and higher values of the specific utilization rates of nitrite compared with that of nitrite (Campos *et al.* 2008; Mora *et al.* 2015).

At steady-state conditions, sulphate constitutes more than 90% of the effluent sulphur compounds while more than 74% and 80% of the influent nitrogen was converted into nitrogen gas in phase II and phase III, respectively. In this context, Equation (3) was used to evaluate the effective impact of autotrophic denitrification process on sulphide removal.

$$HS^- + 0.823\,NO_3^- + 0.177\,HCO_3^- + 0.667\,CO_2$$
$$\rightarrow SO_4^{2-} + 0.169\,C_5H_7NO_2 + 0.003\,H_2O + 0.326\,N_2 \quad (3)$$
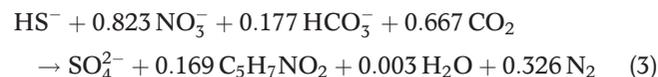
where N/S ratio is 0.36.

The effect of autotrophic denitrification on sulphide removal has been estimated as the difference between the experimentally produced $S_{sulphate}$ (Equation (4)) and the theoretical one (Equation (5)).

$$S_{sulphate,\,measured} = \frac{S\text{-}SO_4^{2-}}{S\text{-}SO_4^{2-} + S_0 + S\text{-}S_2O_3^{2-}} \quad (4)$$

$$S_{sulphate,\,theoretical} = \frac{\Delta N}{(N/S)}\frac{S\text{-}SO_4^{2-}}{S_{tot}} \quad (5)$$

A ratio between $\Delta S_{tot,\,measured}/\Delta S_{tot,theoretical}$ equal to 1.06 and 0.94 resulted in steady-state conditions in phase II and III, respectively.

R2 performance evaluation

Figure 3 shows that, when nitrite alone was used as electron donor (R2) and steady-state conditions were achieved, denitritation reached up to 80% of nitrite removal. In these conditions, thiosulphate partially accumulated in R2, as
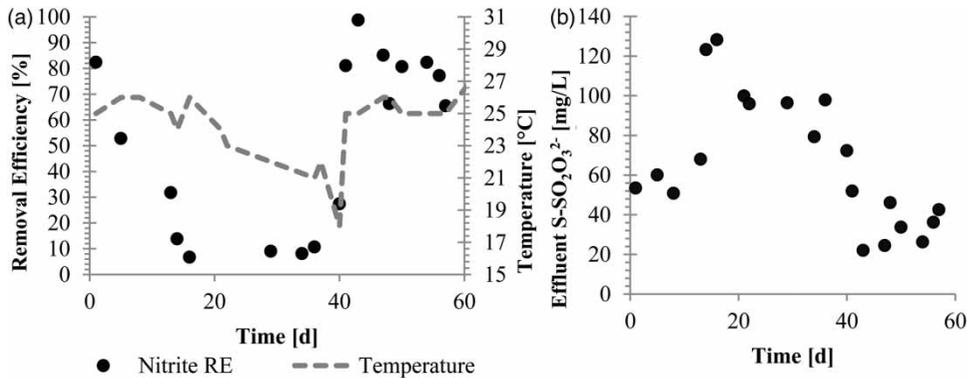
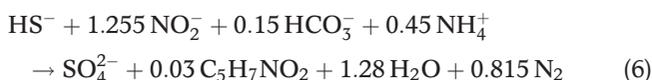**Figure 3** | Nitrite RE (a) and effluent thiosulphate (b) in R2.

observed in R1. Nitrite reduction to nitrogen gas seems to be more sensitive to temperature variations than nitrate reduction to nitrite.

Due to lower nitrite (Krishnakumar & Manilal 1999), nitrate (Chung *et al.* 2014), thiosulphate (Claus & Kutzner 1985; Campos *et al.* 2008) and sulphate (Claus & Kutzner 1985; Oh *et al.* 2000; Campos *et al.* 2008; Chung *et al.* 2014) than threshold values, the effect of gradual and sudden temperature variation seems to be the cause of nitrite and thiosulphate accumulation in both R1 and R2.

Autotrophic denitrification is highly influenced by temperature decrease: N-NO$_2^-$ RE decreases and the increase of soluble chemical oxygen demand (CODsol) indicate an increase in thiosulphate concentration. Thiosulphate production, probably due to chemical processes (Can-Dogan *et al.* 2010), causes a reduction of sulphate in the effluent and an increase of the difference between the sulphate theoretically produced from the oxidation of the total influent sulphide and the measured one. On this basis, it is possible to hypothesize that sulphide oxidation to elemental sulphur and/or thiosulphate takes place with kinetics significantly higher than oxidation of thiosulphate to sulphate; similarly, the reduction of nitrate to nitrite proceeds at higher transformation rates than denitritation.

Sulphur compounds both in the influent and in the effluent of R2 referred to steady-state (from day 45) are reported in Table 2.

The effect of autotrophic denitrification on sulphide removal has been estimated as reported for R1 using Equation (6) (N/S = 0.55).

$$HS^- + 1.255\,NO_2^- + 0.15\,HCO_3^- + 0.45\,NH_4^+$$
$$\rightarrow SO_4^{2-} + 0.03\,C_5H_7NO_2 + 1.28\,H_2O + 0.815\,N_2 \qquad (6)$$

A ratio between $\Delta S_{\text{sulphate, measured}}/\Delta S_{\text{sulphate, theoretical}}$ equal to 0.95 resulted in steady-state conditions.

CONCLUSIONS

The simultaneous sulphur and nitrogen RE through autotrophic denitrification has been tested in operational conditions similar to those that will be obtained from the anaerobic digestion of tannery primary sludge and nitrification of the supernatant.

The results from this study indicate that both denitritation and denitrification with sulphide as electron donor are effective for the removal of sulphide: sulphide RE was higher than 99% in all the tested conditions concerning SRT, temperature and influent S/N ratio.

Sulphide removal kinetics are extremely high, while nitrite tends to be partially accumulated as a function of SRT and T, probably due to sulphide-limiting conditions and slower kinetics of nitrite reduction with respect to nitrate reduction to nitrite. The load of nitrogen present in tannery wastewater is compatible with the application of biogas biological treatment through autotrophic denitrification. In steady-state conditions, nitrogen removal efficiencies higher than 80% were obtained using nitrate or nitrite loads similar to those obtained by nitrification of the supernatant of an anaerobic digester treating vegetable tannery primary sludge.

ACKNOWLEDGEMENTS

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