A novel approach to quantifying elemental sulfur (S^0) in environmental samples

Yu Wang, Feixiang Zan, Gang Guo, Tianwei Hao, Jing Wang and Guanghao Chen

ABSTRACT

The quantification of elemental sulfur (S^0) is an important part of monitoring and controlling sulfur-involving processes. Existing methods of S^0 detection either require significant time or involve the use of toxic chemicals. We have developed and validated a new method to determine S^0 in environmental samples using calorimeter-ion chromatography (IC), in which S^0 is fully oxidized to sulfur trioxide (SO_3) with pure oxygen at 20 atm in a calorimeter. The resulting SO_3 is then absorbed by a sodium bicarbonate ($NaHCO_3$) solution and analyzed using IC. To verify this method, standard samples with various sulfur contents (5–200 mg S), possible interfering substances (SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$ and S^{2-}), and mixed environmental samples were tested and compared. The high correlation of $R^2 = 0.999$ between the examined and theoretical values was obtained with a high recovery rate of $\geq$95% and a low relative standard deviation (RSD) of $\leq$1%. Samples containing at least 25 mg of S^0 were accurately measured (recovery error < 5%). Thiosulfate was identified as the main interfering substance, and pretreatment was needed to eliminate it. This new method is more efficient, cost-effective, easier to operate, and more secure and accurate than existing methods.

Key words | calorimeter, elemental sulfur, ion chromatography, s^0

Yu Wang
Feixiang Zan
Gang Guo
Tianwei Hao (corresponding author)
Jing Wang
Guanghao Chen
Department of Civil & Environmental Engineering,
The Hong Kong University of Science and Technology,
Hong Kong, China
E-mail: *thao@connect.ust.hk*

Tianwei Hao
Institute for Advanced Study,
The Hong Kong University of Science and Technology,
Hong Kong, China
and
Department of Civil and Environmental Engineering, Faculty of Science and Technology,
University of Macau, Macau, China

Guanghao Chen
Hong Kong Branch of Chinese National Engineering Research Center for Control & Treatment of Heavy Metal Pollution,
The Hong Kong University of Science and Technology,
Hong Kong, China
and
Wastewater Treatment Laboratory, FYT Graduate School,
The Hong Kong University of Science and Technology,
Nansha, Guangzhou, China

INTRODUCTION

Elemental sulfur (S^0) is a cost-effective biological water and wastewater treatment agent, serving as an electron donor or acceptor, owing to its widespread availability and low cost (Ashok & Hait 2015; Di Capua *et al.* 2015). S^0 is particularly suited to autotrophic nitrogen removal in potable water and wastewater treatment as it acts as an efficient electron donor, and it can precipitate metal in acid mine drainage treatment by inducing sulfide production (Trouve *et al.* 1998; Dijkman *et al.* 2002; Kaksonen & Puhakka 2007; Sahinkaya *et al.* 2017). However, it is undesirable and potentially harmful in some cases. For instance, deposits of sulfur in sewer sediments and biofilm can cause severe corrosion in sewers through sulfide production (Jiang *et al.* 2009, 2015; Liang *et al.* 2016). The costs of sulfur-induced treatment are in the range of 2–3% of the gross domestic product (GDP) in developed countries (Koch *et al.* 2001). Moreover, the presence of S^0 can reduce the efficiency of methane production in anaerobic digestion systems (Kroiss & Plahl-Wabnegg 1983). As such, quantifying S^0 levels in environmental samples is crucial for monitoring and controlling sulfur-involving processes and/or mitigating its harmful impacts.

Some of the existing methods for quantifying S^0 in environmental samples, such as ion chromatography (IC), spectrophotometry, high-performance liquid chromatography (HPLC), and inductively coupled plasma-optical emission spectroscopy (ICP-OES), are summarized in Table 1. The traditional method using IC involves converting elemental sulfur to thiosulfate at high pH

doi: 10.2166/wst.2018.169

Table 1 | Existing methods for detecting S^0

Methods	Principle	Detection limit	Reference
IC	Detect produced thiosulfate	N/A	Goehring *et al.* (1949), Jiang *et al.* (2009)
Spectrophotometry	Detect produced thiocyanate	5–50 mg/L	Bartlett & Skoog (1954)
HPLC	Detect S^0 directly	4–1,200 μg/g	Rethmeier *et al.* (1997), McGuire & Hamers (2000)
ICP-OES	Detect S^0 directly	N/A	Dutta *et al.* (2008)
Colorimetry	Detect produced sulfide	>0.01 μg/g	Kwasniewski *et al.* (2011)
Voltammetry	Detect S^0 directly	1–50 μM	Rozan *et al.* (2000)

(pH > 12) with sulfite as oxidant and then analyzing thiosulfate by IC to calculate the elemental sulfur concentration (Goehring *et al.* 1949; Jiang *et al.* 2009). Alkaline conditions (pH > 12) and a long oxidation time (>12 hours) are essential in this method, and a low accuracy can be caused by the instability of the thiosulfate produced. The methods using spectrophotometry and colorimetry involve applying some highly toxic compounds (e.g. cyanide or mercuric chloride) (Bartlett & Skoog 1954; Kwasniewski *et al.* 2011), which are not operation-friendly. Although methods using HPLC or ICP-OES typically have high accuracy (Rethmeier *et al.* 1997; Dutta *et al.* 2008), they require expensive instruments (e.g. HPLC or ICP-OES) and complicated pretreatment (e.g. complete digestion or extraction). The voltammetric method proposed by Rozan *et al.* (2000) can discriminate S^0, HS^- and S_x^{2-} in water samples, but whether it is applicable under different conditions has not yet been verified. As a result, a simple, cost-efficient and accurate method for detecting S^0 in environmental samples is desirable.

We have developed a new method of S^0 examination using a coupled 6200 isoperibol calorimeter with IC (calorimeter-IC). Its feasibility and accuracy were validated with a range of different standard sulfur-containing samples, and several possible interfering substances (SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$ and S^{2-}) in the measurements were considered and analyzed. Subsequently, the new method was compared with the existing methods using real environmental samples collected from a biological reactor.

MATERIALS AND METHODS

Reagents and apparatus

All reagents used were of an analytical grade, and solutions were prepared with ultra-pure water. S^0 (purity > 99.5%) was purchased from Damao (Tianjin, China). Sodium bicarbonate ($NaHCO_3$) (99.5–100.5% purity), sodium sulfate (Na_2SO_4) (purity > 99.0%), sodium sulfide nonahydrate ($Na_2S{\cdot}9H_2O$) (purity ≥ 98.0%), sodium thiosulfate ($Na_2S_2O_3$) (99.0% purity), sodium hydroxide (NaOH) (99.0% purity), calcium sulfate ($CaSO_4$) (purity > 99.0%), and carbon were purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium sulfite (Na_2SO_3) (98.3% purity) was purchased from VWR (Radnor, PA, USA). Pure oxygen was obtained from HSG (Hong Kong, China). Ultra-pure water was provided by a Cascada I Integrated Laboratory Water Purification System with an LW32302 final filter (Pall Corporation, Port Washington, NY, USA). An amount of 0.5 mol/L $NaHCO_3$ solution was prepared by dissolving 42 g $NaHCO_3$ in 500 mL ultra-pure water and diluting it to 1,000 mL.

A 6200 isoperibol calorimeter (Parr Instrument Company, Moline, IL, USA) was used to completely combust sulfur samples. It uses a A391DD removable bucket that holds a 342 mL bomb (Parr 1108 oxygen bomb), stirrer, and thermometer, as shown in Figure 1. The pressure of the oxygen bomb was set at 20 atm. An IC (Shimadzu Corporation, Kyoto, Japan; HIC-20A super) equipped with a conductivity detector and an IC-SA2 analytical column was used to analyze the sulfate produced. A freeze dryer (Thermo Fisher Scientific, Waltham, MA, USA) was used to lyophilize water-containing samples (1.5 mTorr; −50 °C).

Procedure

Standard samples with different levels of S^0 (5, 10, 25, 50, 75, 100, 150, 200 mg) were used to validate this method. An overview of the entire procedure is shown in Figure 2. Water-containing samples were lyophilized for 48 hours prior to measurement. The heater of the calorimeter was initially turned on for approximately 20 min to preheat the machine until the jacket temperature reached 30 °C. The weighed sample was then placed on the sample dish with 0.5 g carbon as a combustion improver. After sealing, the oxygen supply was turned on and the bomb was connected to a

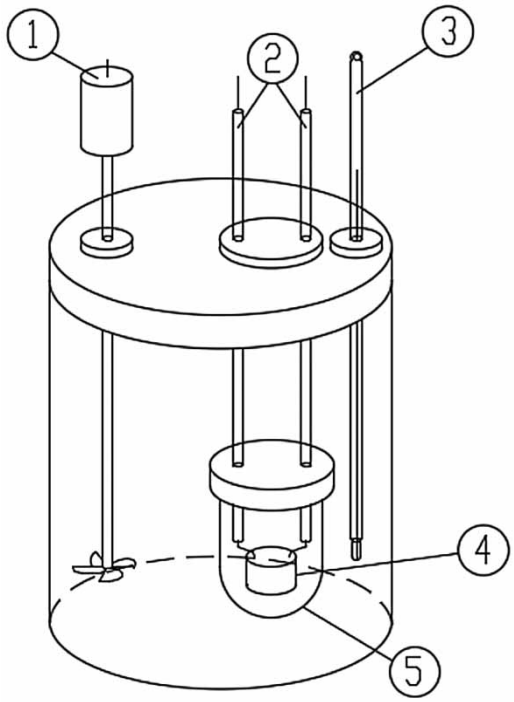

Figure 1 | Schematic diagram of the bucket and bomb: (1) stirrer; (2) ignition wires; (3) thermometer; (4) sample dish; and (5) steel bomb.

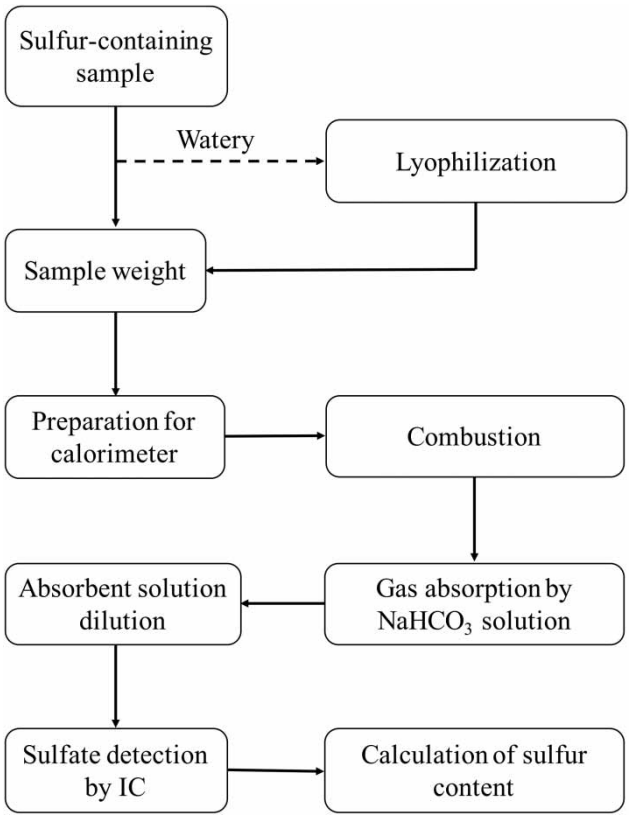

Figure 2 | Flow chart of the sample preparation and examination for S^0 determination.

micro-processor-controlled solenoid valve to fill the bomb with pure oxygen. The sulfur was completely oxidized into sulfur trioxide (SO_3) in the bomb (Equation (1)). After combustion, the compressed gas containing SO_3 in the bomb was slowly released and subsequently absorbed by 0.5 mol/L $NaHCO_3$ solution in a 380 mL two-stage unit. Sulfate ions were produced from SO_3 (Equation (2)). After absorption, the solution was transferred into a volumetric flask and diluted to a final 1,000 mL, and subsequently measured by IC. All of the measurements were conducted in triplicate.

$$2S + 3O_2 \rightarrow 2SO_3(g) \quad (1)$$

$$SO_3 + 2HCO_3^- \rightarrow SO_4^{2-} + H_2O + 2CO_2 \quad (2)$$

Interference study

Reduced inorganic sulfur compounds (RISCs) such as sulfite, thiosulfate, and sulfide can limit the accuracy of this method. Sulfate, which is the final testing objective in this method, is also a potential interfering substance. To evaluate their potential interference, various sulfur-containing samples, namely 25, 50, and 100 mg S of Na_2SO_4 and $CaSO_4$; 25, 50, and 100 mg S of Na_2SO_3 and $Na_2S_2O_3$; and 200, 350, 500, and 800 mg lyophilized Na_2S were tested using this method following the aforementioned procedure.

Analyses of the environmental samples

Several real samples were collected from a biological reactor that recovers sulfur from sulfide with the mediation of using sulfide-oxidizing bacteria. The samples were first lyophilized for 48 hours to reduce moisture and subsequently measured using this method and two other methods (i.e. HPLC and spectrophotometry) for comparison (Bartlett & Skoog 1954; Rethmeier *et al.* 1997).

RESULTS AND DISCUSSION

Measurement of standard samples

The results of the analysis of the standard samples are summarized in Table 2. Relative standard deviations (RSDs) were <1%. The standard deviation (SD) value of each measured sample fell into the narrow range of ≤1.2 mg in every case (i.e. from 5 mg to 200 mg). Samples with a sulfur content of

Table 2 | Measured results for standard sulfur-containing samples

Standard sulfur content (mg)	Mean values and standard deviation (SD) (mg)	Relative standard deviation (RSD) (%, $n = 3$)	Recovery rate (%)
200	191.15 ± 1.20	0.63	95.29
150	149.25 ± 0.32	0.21	99.37
100	96.79 ± 0.48	0.50	96.60
75	75.37 ± 0.68	0.90	100.57
50	47.95 ± 0.09	0.18	95.14
25	26.54 ± 0.23	0.87	104.51
10	13.98 ± 0.02	0.11	138.43
5	8.23 ± 0.08	0.99	158.36

≥25 mg were measured more accurately using this method with a recovery rate ranging from 95.14% to 104.51%, whereas the excess recovery rates of 138.43% and 158.36% were obtained with 10 mg and 5 mg of sulfur, respectively. All of the deviations of the mean values from the theoretical values and RSDs in Table 2 are less than 15%, within the range recommended by the USFDA (2001) for sulfur content exceeding 25 mg, thereby demonstrating the stability, accuracy and precision of this method for analyzing S^0. Methodological shortcomings and investigator errors may have contributed to low-sulfur content samples at 5 and 10 mg S^0. So, increasing the size (volume) of the sample is a reliable and effective method for avoiding potential interference. A sulfur content of at least 25 mg is therefore recommended for this method.

The theoretical (standard) and experimental (observed) values in the 25–200 mg S^0 range, determined with our proposed method, were highly correlated ($R^2 = 0.999$, $n = 8$: Figure 3), highlighting the method's reliability.

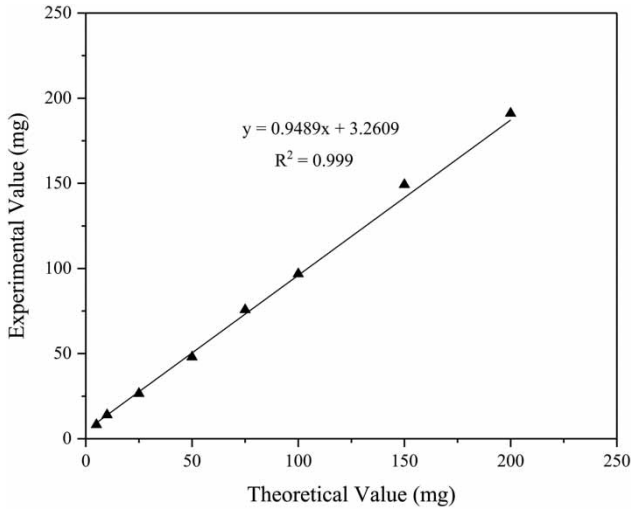

Figure 3 | Linearity between theoretical and experimental values ($n = 8$).

Interference effects of sulfate, sulfite, thiosulfate, and sulfide

Sulfur compounds such as sulfate, sulfite, thiosulfate, and sulfide often appear in environmental samples, and their interference effects must be evaluated to accurately measure S^0. Na_2SO_4 and $CaSO_4$, Na_2SO_3, $Na_2S_2O_3$ and Na_2S were chosen as representatives in this study. Different levels of each representative, i.e. 25, 50, and 100 mg S for sulfate, sulfite, and thiosulfate, respectively, and 200, 350, 500, and 800 mg for sulfide, were examined, and the results are listed in Tables 3 and 4. An interfering concentration generally must to be considered (e.g., proper pretreatment) when an interfering substance causes variations of >5% in the percentage recovery rate (Chen & Li 2014).

As shown in Table 3, less than 10% and 20% of sulfur can be detected in Na_2SO_4 and $CaSO_4$, respectively. The recovery rates of sulfur in Na_2SO_4 were 9.35% for 25 mg S, 5.54% for 50 mg S, and 2.95% for 100 mg S. Higher recovery rates of 11.65–17.82% were recorded for $CaSO_4$, possibly due to the release of sulfur dioxide in the presence of carbon (Talukdar *et al.* 1996). The mass ratio of S^0 over sulfate in the sample is a critical factor and the interference is significant at low ratios. This indicated that when the ratios of S^0 over Na_2SO_4 and S^0 over $CaSO_4$, in terms of sulfur in the samples, are less than 2 and 4, respectively, sulfate compounds must be avoided by

Table 3 | Sulfate, sulfite, and thiosulfate interference examination

Content / Species	Measurement mean and SD (mg S)			RSD (%, $n = 3$)			Recovery rate (%)		
	100	50	25	100	50	25	100	50	25
Na_2SO_4	2.95 ± 0.10	2.77 ± 0.01	2.34 ± 0.06	3.30	0.40	2.56	2.95	5.54	9.35
$CaSO_4$	11.65 ± 0.01	6.52 ± 0.01	4.45 ± 0.02	0.07	0.16	0.34	11.65	13.03	17.82
Na_2SO_3	2.39 ± 0.02	1.82 ± 0.08	1.29 ± 0.04	0.80	4.21	3.24	2.39	3.62	5.14
$Na_2S_2O_3$	80.04 ± 0.12	34.83 ± 0.06	18.62 ± 0.04	0.15	0.17	0.23	80.10	69.62	74.34

Table 4 | Sulfide interference examination

Sulfide content (mg S)	Mean and SD (mg S)	RSD (%, $n = 3$)	Recovery rate (%)
312.82	31.54 ± 1.03	3.27	10.08
195.62	19.78 ± 0.75	3.78	10.11
136.90	12.40 ± 0.10	0.79	9.06
78.37	10.29 ± 0.19	1.80	13.14

dissolving and then filtering the samples prior to the determination to reduce sulfate interference.

Small amounts of sulfur in Na_2SO_3 (recovery rate <5.14%) were detected with sulfite content of 25–100 mg S. The recovery rates of sulfur in Na_2SO_3 were only around 5% (i.e. 5.14% for 25 mg S, 3.62% for 50 mg S and 2.39% for 100 mg S), whereas the majority of sulfur in $Na_2S_2O_3$ was measured, yielding the recovery rates of 74.34%, 69.62%, and 80.10% for $Na_2S_2O_3$ levels of 25, 50, and 100 mg S, respectively. This may be due to the oxidation of sulfur atoms with valence of −2 or 0 in thiosulfate anions (Vairavamurthy *et al.* 1993; Suzuki 1999). When the mass ratio of S^0 over $Na_2S_2O_3$, in terms of sulfur, is less than 16, pretreatment such as abstersion prior to determination can effectively prevent the interference of $Na_2S_2O_3$.

Elemental sulfur and sulfate are the oxidized products of sulfide under natural conditions. $Na_2S{\cdot}9H_2O$ was therefore used for the investigation of sulfide interference. After the lyophilizing treatment, the Na_2S content in the sample was 95.23% (i.e. 1 g sample contained 0.9523 g Na_2S). Therefore, the sulfur content in lyophilized samples were 78.37, 136.90, 195.62, and 312.82 mg S for 200, 350, 500, and 800 mg, respectively. As shown in Table 4, the recovery rates of sulfur in Na_2S were 13.14% for 200 mg, 9.06% for 350 mg, 10.11% for 500 mg, and 10.08% for 800 mg. So when the ratio of S^0 over Na_2S, in terms of sulfur in the sample, is less than 3, pretreatment such as abstersion is needed to eliminate the interference of Na_2S.

Environmental sample test and comparison

Our new approach was compared with existing methods (i.e. spectrophotometry and HPLC) using real environmental samples. Three composite samples were obtained from nine samplings in a biological sulfide oxidation reactor under different operational periods and mixed in equal proportions after the lyophilizing treatment. The results obtained using these three methods are shown in Table 5. Compared with the values obtained from our new approach, the relative errors were lower than 2.3%, except for the case of sample 1 using HPLC with a relative error of 6.6%. For instance, the percentages of

Table 5 | Comparison of S^0 content examined by HPLC, spectrophotometry and the proposed calorimeter-IC

S^0 content (%) / Method	Sample 1	Sample 2	Sample 3
HPLC	45.88 ± 1.98	71.85 ± 3.13	75.71 ± 2.45
Spectrophotometer	49.78 ± 0.89	71.99 ± 2.05	74.16 ± 1.93
This method	49.12 ± 0.16	72.68 ± 0.15	74.02 ± 0.36

S^0 in sample 2 were 71.85%, 71.99%, and 72.68% for HPLC, spectrophotometry, and this method, respectively, and the SD of this method was 0.15%, which is significantly lower than that of the HPLC (SD = 3.13%) or spectrophotometry method (SD = 2.05%). To further demonstrate the reliability of the results, a Friedman test was conducted with a significance level of 0.717 at 95% confidence intervals, suggesting no statistically significant difference in these results. This demonstrates the coherence of these three methods and further highlight the feasibility and authenticity of our new method.

The cost of the reagent consumed per sample is <US$0.5 with this method, which is more cost effective than the existing methods, for instance when using colorimetry (~US$10/run), as reported by Kwasniewski *et al.* (2011), HPLC (~US$8/run) and spectrophotometer (~US$5/run) (estimated based on the experiments in our laboratory). And the capital cost of the calorimeter is around one-third the capital cost of HPLC and half the capital cost of ICP-OES. In addition, the duration of this method can be shortened to a minimum of 3 hours, which is time-saving if compared with the other existing methods such as the method proposed by Goehring *et al.* (1949) (the IC method lasts more than 12 hours). Moreover, the proposed calorimeter-IC method in this study has the advantages of: (1) ease of operation and preparation; (2) low cost; (3) highly secure reagents; and (4) relatively high accuracy and precision. This study provides a new S^0 examination method that can be easily applied in the environmental field, particularly in the processes of sulfur transformation, utilization, recovery, and removal.

CONCLUSIONS

In this study, a new approach to quantifying S^0 in environmental samples using calorimeter-IC was developed and validated. The main advantages of this method are that it is easy to operate and cost-effective as well as being accurate, repeatable and secure, making it possible to satisfactorily determine S^0 in environmental samples. Thus, this method

can be used with sulfur-involving processes, such as the bioreactors, anaerobic digestion systems and sewers to better control the impacts of S^0. $Na_2S_2O_3$ was identified as the main interfering substance requiring pretreatment such as abstersion. The critical S^0 content in a sample for the calorimeter-IC method is 25 mg with high recovery (recovery error < 5%) and RSD (<1%). More studies must be done to improve the detection range, particularly for samples that have a S^0 content less than 25 mg.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (51638005), the Research Grants Council (RGC) of Hong Kong (Project 6214117) and Guangdong Provincial Science and Technology Planning Project (grant no. 2016A050503041).

REFERENCES

Ashok, V. & Hait, S. 2015 Remediation of nitrate-contaminated water by solid-phase denitrification process – a review. *Environmental Science and Pollution Research* **22** (11), 8075–8093.

Bartlett, J. K. & Skoog, D. A. 1954 Colorimetric determination of elemental sulfur in hydrocarbons. *Analytical Chemistry* **26** (6), 1008–1011.

Chen, Z. & Li, H. 2014 A method of high sensitivity and in situ determination of trace cobalt (II) in water samples with salicyl fluorone. *Water Science and Technology* **70** (7), 1182–1187.

Di Capua, F., Papirio, S., Lens, P. N. & Esposito, G. 2015 Chemolithotrophic denitrification in biofilm reactors. *Chemical Engineering Journal* **280**, 643–657.

Dijkman, H., Boonstra, J., Lawrence, R. & Buisman, C. J. 2002 Optimization of metallurgical processes using high rate biotechnology. *Sulfide Smelting* 113–123.

Dutta, P. K., Rabaey, K., Yuan, Z. & Keller, J. 2008 Spontaneous electrochemical removal of aqueous sulfide. *Water Research* **42** (20), 4965–4975.

Goehring, M., Feldmann, U. & Helbing, W. 1949 Quantitative bestimmung der polythionate (trithionat, tetrathionat, pentathionat und hexathionat) nebeneinander (Quantitative determination of the polythionates (trithionate, tetrathionate, pentathionate and hexathionate) side by side). *Fresenius' Journal of Analytical Chemistry* **129** (4), 346–352.

Jiang, G., Sharma, K. R., Guisasola, A., Keller, J. & Yuan, Z. 2009 Sulfur transformation in rising main sewers receiving nitrate dosage. *Water Research* **43** (17), 4430–4440.

Jiang, G., Sun, J., Sharma, K. R. & Yuan, Z. 2015 Corrosion and odor management in sewer systems. *Current Opinion in Biotechnology* **33**, 192–197.

Kaksonen, A. H. & Puhakka, J. A. 2007 Sulfate reduction based bioprocesses for the treatment of acid mine drainage and the recovery of metals. *Engineering in Life Sciences* **7** (6), 541–564.

Koch, G., Kühni, M., Rieger, L. & Siegrist, H. 2001 Calibration and validation of an ASM3–based steady-state model for activated sludge systems – part II: prediction of phosphorus removal. *Water Research* **35** (9), 2246–2255.

Kroiss, H. y. & Plahl-Wabnegg, F. 1983 Sulphide toxicity with anaerobic wastewater treatment. In: *Environmental Symposium on Anaerobic Wastewater Treatment, The Netherlands*, pp. 72–85.

Kwasniewski, M. T., Allison, R. B., Wilcox, W. F. & Sacks, G. L. 2011 Convenient, inexpensive quantification of elemental sulfur by simultaneous in situ reduction and colorimetric detection. *Analytica Chimica Acta* **703** (1), 52–57.

Liang, S., Zhang, L. & Jiang, F. 2016 Indirect sulfur reduction via polysulfide contributes to serious odor problem in a sewer receiving nitrate dosage. *Water Research* **100**, 421–428.

McGuire, M. M. & Hamers, R. J. 2000 Extraction and quantitative analysis of elemental sulfur from sulfide mineral surfaces by high-performance liquid chromatography. *Environmental Science & Technology* **34** (21), 4651–4655.

Rethmeier, J., Rabenstein, A., Langer, M. & Fischer, U. 1997 Detection of traces of oxidized and reduced sulfur compounds in small samples by combination of different high-performance liquid chromatography methods. *Journal of Chromatography A* **760** (2), 295–302.

Rozan, T. F., Theberge, S. M. & Luther, G. 2000 Quantifying elemental sulfur (S0), bisulfide (HS−) and polysulfides (Sx2−) using a voltammetric method. *Analytica Chimica Acta* **415** (1), 175–184.

Sahinkaya, E., Yurtsever, A. & Ucar, D. 2017 A novel elemental sulfur-based mixotrophic denitrifying membrane bioreactor for simultaneous Cr (VI) and nitrate reduction. *Journal of Hazardous Materials* **324**, 15–21.

Suzuki, I. 1999 Oxidation of inorganic sulfur compounds: chemical and enzymatic reactions. *Canadian Journal of Microbiology* **45** (2), 97–105.

Talukdar, J., Basu, P. & Greenblatt, J. H. 1996 Reduction of calcium sulfate in a coal-fired circulating fluidized bed furnace. *Fuel* **75** (9), 1115–1123.

Trouve, C., Chazal, P. M., Gueroux, B. & Sauvaitre, N. 1998 Denitrification by new strains of *Thiobacillus denitrificans* under non-standard physicochemical conditions. Effect of temperature, pH, and sulphur source. *Environmental Technology* **19** (6), 601–610.

US Food and Drug Administration 2001 *FDA Guidance for Industry: Bioanalytical Method Validation*. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD.

Vairavamurthy, A., Manowitz, B., Luther, G. W. & Jeon, Y. 1993 Oxidation state of sulfur in thiosulfate and implications for anaerobic energy metabolism. *Geochimica et Cosmochimica Acta* **57** (7), 1619–1623.

First received 15 August 2017; accepted in revised form 3 April 2018. Available online 12 April 2018