A novel approach to quantifying elemental sulfur (S0) in environmental samples
Yu Wang, Feixiang Zan, Gang Guo, Tianwei Hao, Jing Wang and Guanghao Chen

ABSTRACT

The quantification of elemental sulfur (S0) is an important part of monitoring and controlling sulfur-involving processes. Existing methods of S0 detection either require significant time or involve the use of toxic chemicals. We have developed and validated a new method to determine S0 in environmental samples using calorimeter-ion chromatography (IC), in which S0 is fully oxidized to sulfur trioxide (SO3) with pure oxygen at 20 atm in a calorimeter. The resulting SO3 is then absorbed by a sodium bicarbonate (NaHCO3) solution and analyzed using IC. To verify this method, standard samples with various sulfur contents (5–200 mg S), possible interfering substances (SO42−/CO32−, SO32−/CO32−, S2O32−/CO32− and S2−/CO32−), and mixed environmental samples were tested and compared. The high correlation of R2 = 0.999 between the examined and theoretical values was obtained with a high recovery rate of ≥95% and a low relative standard deviation (RSD) of ≤1%. Samples containing at least 25 mg of S0 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, S0

INTRODUCTION

Elemental sulfur (S0) 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). S0 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 S0 can reduce the efficiency of methane production in anaerobic digestion systems (Kroiss & Plahl-Wabnegg 1985). As such, quantifying S0 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 S0 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.
(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; McGuire & Hamers 2000), 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 S0, HS- and S2- 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 S0 in environmental samples is desirable.

We have developed a new method of S0 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 (SO42-, SO32-, S2O32- and S2-) 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. S0 (purity > 99.5%) was purchased from Damao (Tianjin, China). Sodium bicarbonate (NaHCO3) (99.5–100.5% purity), sodium sulfate (Na2SO4) (purity > 99.0%), sodium sulfide nonahydrate (Na2S·9H2O) (purity ≥ 98.0%), sodium thiosulfate (Na2S2O3) (99.0% purity), sodium hydroxide (NaOH) (99.0% purity), calcium sulfate (CaSO4) (purity > 99.0%), and carbon were purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium sulfite (Na2SO3) (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 NaHCO3 solution was prepared by dissolving 42 g NaHCO3 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 A391IDD 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 S0 (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

<table>
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<td>IC</td>
<td>Detect produced thiosulfate</td>
<td>N/A</td>
<td>Goehring et al. (1949), Jiang et al. (2009)</td>
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<td>Spectrophotometry</td>
<td>Detect produced thiocyanate</td>
<td>5–50 mg/L</td>
<td>Bartlett &amp; Skoog (1954)</td>
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<td>HPLC</td>
<td>Detect S0 directly</td>
<td>4–1,200 μg/g</td>
<td>Rethmeier et al. (1997), McGuire &amp; Hamers (2000)</td>
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<tr>
<td>ICP-OES</td>
<td>Detect S0 directly</td>
<td>N/A</td>
<td>Dutta et al. (2008)</td>
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<tr>
<td>Colorimetry</td>
<td>Detect produced sulfide</td>
<td>&gt;0.01 μg/g</td>
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<td>Voltammetry</td>
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micro-processor-controlled solenoid valve to fill the bomb with pure oxygen. The sulfur was completely oxidized into sulfur trioxide (SO₃) in the bomb (Equation (1)). After combustion, the compressed gas containing SO₃ in the bomb was slowly released and subsequently absorbed by 0.5 mol/L NaHCO₃ solution in a 380 mL two-stage unit. Sulfate ions were produced from SO₃ (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 + 3O₂ → 2SO₃(g)  \hspace{1cm} (1)

SO₃ + 2HCO₃⁻ → SO₄²⁻ + H₂O + 2CO₂  \hspace{1cm} (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 Na₂SO₄ and CaSO₄; 25, 50, and 100 mg S of Na₂SO₃ and Na₂S₂O₃; and 200, 350, 500, and 800 mg lyophilized Na₂S 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
≥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 S0.

**Methodological shortcomings and investigator errors may have contributed to low-sulfur content samples at 5 and 10 mg S0, 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.**

Sulfur compounds such as sulfate, sulfite, thiosulfate, and sulfide often appear in environmental samples, and their interference effects must be evaluated to accurately measure S0. Na2SO4 and CaSO4, Na2SO3, Na2S2O3 and Na2S 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 Na2SO4 and CaSO4, respectively. The recovery rates of sulfur in Na2SO4 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 CaSO4, possibly due to the release of sulfur dioxide in the presence of carbon (Talukdar et al. 1996). The mass ratio of S0 over sulfate in the sample is a critical factor and the interference is significant at low ratios. This indicated that when the ratios of S0 over Na2SO4 and S0 over CaSO4, in terms of sulfur in the samples, are less than 2 and 4, respectively, sulfate compounds must be avoided by...
dissolving and then filtering the samples prior to the determination to reduce sulfate interference.

Small amounts of sulfur in Na$_2$SO$_3$ (recovery rate <5.14%) were detected with sulfite content of 25–100 mg S. The recovery rates of sulfur in Na$_2$SO$_3$ 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$_2$S$_2$O$_3$ was measured, yielding the recovery rates of 74.34%, 69.62%, and 80.10% for Na$_2$S$_2$O$_3$ 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$_2$S$_2$O$_3$, in terms of sulfur, is less than 16, pretreatment such as abstersion prior to determination can effectively prevent the interference of Na$_2$S$_2$O$_3$.

Elemental sulfur and sulfate are the oxidized products of sulfide under natural conditions. Na$_2$S·9H$_2$O was therefore used for the investigation of sulfide interference. After the lyophilizing treatment, the Na$_2$S content in the sample was 95.23% (i.e. 1 g sample contained 0.9523 g Na$_2$S). 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 S, respectively. As shown in Table 4, the recovery rates of sulfur in Na$_2$S were 13.14% for 200 mg, 9.06% for 350 mg, 10.11% for 500 mg, and 10.08% for 800 mg S. So when the ratio of $S^0$ over Na$_2$S, in terms of sulfur in the sample, is less than 3, pretreatment such as abstersion is needed to eliminate the interference of Na$_2$S.

### 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 $S^0$ 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 main interfering substance requiring pretreatment such as error have a $S^0$ content less than 25 mg. improve the detection range, particularly for samples that have a $S^0$ content less than 25 mg.

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