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Performance evaluation of combining microaerobic desulfurization with addition of rusty scrap iron (RSI) during waste-activated sludge digestion

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ABSTRACT

In this study, microaerobic was integrated into rusty scrap iron (RSI)-loaded anaerobic digester, which has been experimentally demonstrated to improve digestion performance and to decrease biogas H_2S content during waste-activated sludge digestion. A series of batch experiments were carried out, determining that an RSI dosage of 20 g/L was optimal for anaerobic digestion, average methane yield increased by 39.9% and H_2S content decreased by 84.6%. Under optimal RSI dosage conditions, increasing O_2 levels were added stepwise seven stages in a semi-continuous experiment. O_2 addition induced the microbial oxidation of sulfide by stimulating sulfur-oxidizing bacteria and chemical corrosion of iron, which promoted generation of Fe(II) and Fe(III), which subsequently precipitated as FeS and Fe_2S_3. In the sixth period of semi-continuous experiment, deep desulfurization was reached without negatively impacting system performance. Average methane yield was 301.1 mL/g chemical oxygen demand, H_2S concentration was 75 parts per million by volume (ppmv) and desulfurization efficiency reached 99.40%. Sulfur mass balance was described for the system, with 84.0%, 11.90% and 0.21% of sulfur present in solid, liquid and gaseous phases, respectively. These results demonstrated that RSI addition in combination with microaeration represents a promising method for simultaneous controlling biogas H_2S concentration and improving digestion performance.

Keywords: Microaerobic; Sulfide; Average methane yield; Desulfurization efficiency; Sulfur balance

1. Introduction

As the by-product of the wastewater treatment plants the output of waste-activated sludge generated in biological wastewater treatment processes has increased continuously in the recent decade. Wet sludge production in China was estimated to reach 33.59 million ton (based on moisture content 80%) by the end of 2015. Activated sludge has a complex composition, containing a variety of bacteria and organic materials, and improper disposal and accumulation is bound to cause secondary pollution.

Anaerobic treatment process has been widely applied to the treatment of organic solid waste due to low operational costs and high solids reduction efficiency [1–3]. Under anaerobic conditions, organic matter is initially hydrolyzed and then fermented into volatile fatty acids such as acetic acid, as well as hydrogen, which represent substrates for methanogenic archaea for methane production [4]. However, the application of anaerobic digestion of sludge is often limited by low methane yield and sludge reduction rates [5], and the limiting factors are generally associated with the slow

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hydrolysis of sludge. To accelerate the sludge digestion, various pretreatments have been used to improve the hydrolysis of the sludge, including thermal [6], chemical [7] and mechanical methods [8]. The operating cost of the present pretreatment is high and often unattractive for practical application.

Zero-valent iron (ZVI), which is a cheap reductant, has been widely applied to accelerate hydrolysis–acidification of the anaerobic digestion of sludge [9,10]. ZVI addition is associated with a resulting decline in oxidation–reduction potential (ORP) when added into anaerobic systems, enabling a more favorable environment for anaerobic biological processes [11]. Rusty scrap iron (RSI) is an abandoned iron material and covers a layer of iron oxide (rust) on surface, which may represent an economic alternative. However, in previous work, little research has investigated the effect of RSI on anaerobic digestion of activated sludge.

The issue of the quality and quantity of biogas is equally important. Biogas contains several pollutants formed during the anaerobic digestion of sludge, predominantly in the form of toxic hydrogen sulfide (H_2S), with concentrations ranging from 0.1% to 1.0% v/v (1,000–10,000 ppmv) [12]. H_2S can be released during the anaerobic digestion process by specific microorganisms such as sulfate-reducing bacteria (SRB), due to the existence of sulfur-containing compounds in substrates [13,14]. This leads to many problems, such as inhibited anaerobic digestion process, reduced biogas production and poor biogas quality [15]. Consequently, H_2S production must be prevented, or H_2S must be removed from the biogas.

Recently, biological treatment processes to eliminate hydrogen sulfide have been shown to lower operational costs compared with traditional physicochemical processes and lower chemical utilization or eliminate it altogether [16]. Biological removal is based on the utilization of sulfur-oxidizing bacteria (SOB) able to metabolize hydrogen sulfide to obtain energy when oxygen is present as an electron acceptor. The biological oxidation takes place in stages, through several redox intermediates as shown in Eq. (1) [17]. The main reactions carried out by SOB are shown below. At this point, it should be noted that H_2S oxidation in biological systems occurs concurrently with chemical reactions [18].

$$HS^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_4^{2-} \quad (1)$$

$$H_2S + 0.5O_2 \rightarrow S^0 + H_2O;\ \Delta G^\circ = -209.4\ \text{kJ/reaction} \quad (2)$$

$$S^0 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+;\ \Delta G^\circ = -587.41\ \text{kJ/reaction} \quad (3)$$

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+;\ \Delta G^\circ = -798.2\ \text{kJ/reaction} \quad (4)$$

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+;\ \Delta G^\circ = -818.3\ \text{kJ/reaction} \quad (5)$$

Therefore, in this work, the effect of RSI on anaerobic digestion of activated sludge is explored. In addition to this, the effect of a limited oxygen supply to the RSI-loaded anaerobic digester's performance and biogas desulfurization is evaluated.

2. Materials and methods

2.1. Substrates and inoculant and rusty scrap iron

The waste-activated sludge (WAS) used in this study originated from Jianning Economic Development Zone WWPT of Nanjing, China. The sludge was stored in the freezer at –20°C until use. In order to strengthen the hydrolysis step, the sludge was pretreated using alkaline method before the anaerobic fermentation [19]. In brief, the pH of sludge was adjusted to 12 using 4 mol/L of sodium hydroxide, and then the sludge was stirred at 80 rpm for 6 h. After pretreatment, the pH of sludge was adjusted to 7 using 4 mol/L of hydrochloric acid for anaerobic digestion. The characteristics of dewatered sludge and alkaline-pretreated sludge are compared in Table 1.

Inoculated anaerobic microorganisms were at the concentration of total solids (TS) = 34.7 g/L (volatile solids (VS) = 24.1 g/L), which were collected from the expanded granular sludge bed at a wastewater treatment plant for Jiangsu Yanghe Brewery Joint-Stock Co., Ltd. (Suqian City of Jiangsu Province in China). Prior to use, the inocula were starved for 1 week.

RSI was used for addition of ferro-oxidative material for this study. The RSI tailings (approximately 10 mm × 10 mm × 0.3 mm) were obtained from a Machine Processing Factory workshop. The RSI had a layer of corrosion covering the surface of the scrap, and was soaked in 0.1 mol/L of NaOH solution for 24 h to remove oil residue and then washed with deionized water.

2.2. Experimental procedures

2.2.1. Batch anaerobic digestion

In order to examine the effects of varying concentrations of RSI additions (i.e., 0, 1, 5, 10, 20, 30 g/L) on anaerobic digestion performance from WAS, and to obtain the optimal dosage

Table 1
The characteristics of waste activated sludge (WAS) and alkaline-pretreated sludge (APS)

Parameters	WAS	APS
TCOD (total chemical oxygen demand), mg/L	31,253 ± 4,107	30,854 ± 4,494
SCOD (soluble chemical oxygen demand), mg/L	121 ± 25	721 ± 28
TS (total solids), g/L	33.6 ± 4.9	32.9 ± 4.2
VS (volatile solids), g/L	20.7 ± 2.7	20.3 ± 2.4
TSS, g/L	32.4 ± 4.3	31.5 ± 3.9
VSS, g/L	19.8 ± 2.1	19.2 ± 1.8
pH	7.16 ± 0.11	7.06 ± 0.04
Total Fe, mg/L	31.2 ± 0.97	31.2 ± 0.94
Soluble Fe, mg/L	8.7 ± 0.15	9.4 ± 0.16
Soluble sulfide, mg/L	34.7 ± 1.5	43.9 ± 1.8
SO_4^{2-}, mg/L	3.4 ± 0.18	5.5 ± 0.22
Total elemental sulfur[a], mg/L	279.6 ± 13.1	279.6 ± 11.8

[a]Total elemental sulfur: total sulfur as sum of S in all sulfur compounds.

of RSI for the subsequent semi-continuous anaerobic digestion experiment, a series of batch trials were carried out. The experiments were carried out in lab-scale glass reactors with a total volume of 500 mL and a working volume of 400 mL with silica gel stoppers, which were filled with 100 mL of seed sludge and 300 mL of feed sludge. The volumetric organic loading rate of the reactors was 15.2 g·VS/(L·d). The ratio of inoculum to substrate (on VS basis) was 0.40. Nitrogen gas was injected into each bottle for 5 min to remove the air. The experiments were operated under mesophilic condition (35°C) in a shaking incubator at 150 rpm, which were operated for 30 d. The initial pH of these reactors was adjusted to 7.2 and monitored without adjustment once a day in later. Biogas generated was collected in a 5 L Tedlar gas bag (CEL Scientific, Santa Fe Springs, CA, USA). Sludge was sampled every day and conserved at 4°C. To increase the amount of hydrogen sulfide produced during digestion and simulate waste streams with significant sulfate loading, 0.5 g sodium sulfate was added to the reactors every day. Each trial was carried out in triplicate.

2.2.2. Semi-continuous microaerobic digestion

To investigate the effects of a limited oxygen supply on SOB in an RSI-loaded anaerobic digester's performance and desulfurization, lab-scale bioreactors were operated with a working volume of 4 L and a headspace of 0.5 L under varying oxygen dosages and the optimal dosage of RSI as determined above over a span of 210 d. Digestion was performed in the mesophilic range (35°C ± 1°C) with a hydraulic retention time of approximately 20 d. To increase the amount of hydrogen sulfide produced during digestion, sodium sulfate was added to the feed at a concentration of 2 g/L. The bioreactor was fed with WAS through a peristaltic pump on a semi-continuous basis, with feeding and discharge once a day (200 mL). The reactor was mixed with biogas recirculation provided by a miniature electroAD compressor (1 L/min). Oxygen was supplied with a SIERRA 820 Top-Trak mass flow controller from an oxygen cylinder. The oxygen was introduced into the headspace of reactor once a day. The study was divided into seven operational periods with systematically increased dosages of oxygen. According to the relationship of 5.0 NL oxygen/Nm^3 biogas and the biogas production in P2 to determine the dosage of oxygen (about 0.6–12.0 NL oxygen/Nm^3 biogas). The first period (P1) represented a control, without RSI added in the absence of oxygen to establish performance of conventional anaerobic digestion of WAS, as determined by chemical oxygen demand (COD) removal and methane yield. The second period (P2) investigated the effect of addition of the optimally determined RSI concentration on anaerobic digestion performance in comparison with P1. The five additional periods (i.e., P3–P7) were carried out to evaluate the effect of the varying dosages of oxygen supply on the RSI-loaded anaerobic digester's performance and desulfurization in this system. Table 2 summarizes the experimental arrangement of the study, and a lab-scale bioreactor diagram is shown in Fig. S1. Reactor contents were sampled every day and conserved at 4°C. Biogas generated was collected in a 5 L Tedlar gas bag (CEL Scientific, Santa Fe Springs, CA, USA). Considering the amount of biogas yet to be treated, a biogas residence time (BRT) of more than 7 h would be maintained, which was sufficient to obtain an efficient hydrogen sulfide removal in a microaerobic reactor [20].

2.3. Analysis

Prior to daily sampling, pH and ORP values were quantified by HQ30d meter equipped with a standard electrode (PHC101) and a standard electrode (MTC101), respectively. And the value of ORP expressed in relation to the hydrogen electrode in accordance with the standard procedure—ORPH (Eh). TS, VS, total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were measured according to the standard methods [21]. For analysis of the soluble fraction, the samples were centrifuged at 8,000 rpm for 10 min and then the supernatant was passed through a nitrocellulose 0.45 mm filter.

Fe(II) and Fe(T) [Fe(II) + Fe(III)] were examined according to standard methods [20]. Samples for Fe(II) analysis were acidified by 0.1 M HCl and measured immediately to minimize its oxidation. Aqueous concentrations were determined after filtering water samples through 0.22 μm glass-fiber filter papers (Gelman A/E, Ann Arbor, USA).

Table 2
Experimental parameters and results during semi-continuous anaerobic and microaerobic digestion of activated sludge supplemented with rusty scrap iron (RSI) over seven periods of operation associated with stepwise increases in oxygen concentration

Period	P1	P2	P3	P4	P5	P6	P7
Condition	An[a]	An	Ma[b]	Ma	Ma	Ma	Ma
Day	1–30	31–60	61–90	91–120	121–150	151–180	181–210
Duration, d	30	30	30	30	30	30	30
RSI dosages, g/L	0	20	20	20	20	20	20
Average biogas production rate, mL/d	937	1,671	1,645	1,592	1,644	1,591	1,197
O_2 supply, mL	0	0	1	5	10	15	20
O_2/biogas[c]	0	0	0.61	3.14	6.08	9.43	16.71
BRT, h	12.8	7.1	7.2	7.5	7.2	7.5	10.0

[a]An, anaerobic.
[b]Ma, microaerobic.
[c]O_2/biogas, ratio of supply of oxygen to biogas produced from anaerobic digestion at standard condition (NL oxygen/Nm^3 biogas).

Sulfate and thiosulfate were measured by HPLC according to the method described by van der Zee et al. [18]. Total sulfide in the effluent was analyzed by the potentiometric titration method (Leici PHS-3C, Shanghai, China), which included H_2S, HS^- and S^{2-}. The sulfur content in the sludge was estimated from an elemental analysis of sulfur. Samples were dried at 95°C, cooled and analyzed in a LECO SC32 oven. Analysis was carried out by combustion of the samples at 1,350°C, as a result, the sulfur was completely oxidized to SO_2 and evaluated in the detection cell. The sulfur estimation was the result of subtracting the content of the other analyzed sulfur species (sulfate, thiosulfate) of the sample from the total sulfur content.

Biogas composition (CH_4, CO_2, H_2S and O_2) was measured daily using a portable biogas analyzer (Biogas 5000, Geotech, UK). The gas bag was emptied at the end of each working day, with biogas accumulated over 24 h, with biogas samples taken for analysis at the beginning of the following day. The volume was measured by displacement of water then converted to STP (standard temperature and pressure).

3. Results and discussion

3.1. Sludge digestion system with RSI amendments during batch anaerobic digestion

3.1.1. The variation of methane yield and methane content

The effects of RSI on the anaerobic WAS digestion system were evaluated by characterizing variations in cumulative methane yield, methane content, VS reduction and concentration of hydrogen sulfide in biogas. Fig. 1(a) illustrates cumulative methane yield profiles under different RSI dosages in batch digestion. As can be seen, supply of RSI substantially promoted methane production, consistent with observations by Zhang et al. [22]. Moreover, as RSI additions increased, so too did the methane generation. For example, with an increase of RSI from 0 (RSI-free) to 1 g/L, methane yield exhibited a progressive climb from 135.4 ± 1.5 to 141.9 ± 2.6 mL/gVS fed, which was further enhanced as RSI increased to 5 g/L and above. The highest methane production of 210.7 ± 6.5 mL/gVS fed was obtained at 30 g-RSI/L, 55.6% higher relative to the RSI-free. In terms of methane content, distinct differences were also observed circumstances of increasing RSI. As can be seen in Fig. 1(b), methane content at 0 g-RSI/L was quite low and stable at 51.2%–56.4% between day 9 and day 30, which further rose to 61.1%–71.2% and then to 61.9%–71.4% as RSI elevated to 20 and 30 g/L. Methane content achieved at the optimum RSI of 30 g/L in this experiment was comparably higher than that of acid pretreated sludge (54.2% ± 2.3%) [23], alkaline-ultrasound pretreated sludge (64%–70%) [24], sono-alkalization pretreated sludge (57.6%–62.1%) [25] or ZVI-sludge (62.0%) [26].

3.1.2. Sludge reduction

Biogas is recognized to be produced through the conversion of organic matter in sludge by anaerobic microorganisms. Based on mass balance calculations, the removal of VS is illustrated in Fig. 2, wherein removal rates for VS were obviously affected by the supplementation of RSI in the sludge digestion system. Average VS removal rates of 22.3%, 24.6%, 29.2%, 35.5%, 40.7% and 41.6% were achieved with RSI dosages of 0, 1, 5, 10, 20 and 30 g/L, respectively, suggesting a general

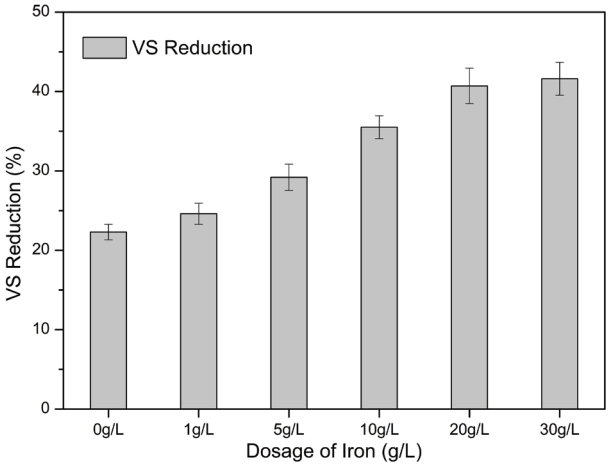

Fig. 2. Effect of RSI dosages on VS reduction during batch digestion of activated sludge.

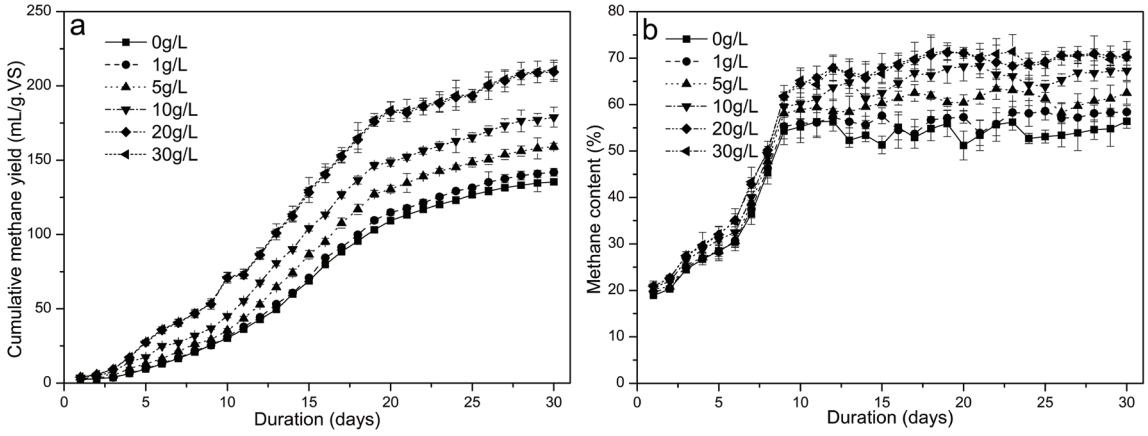

Fig. 1. Effect of RSI dosages on CH_4 production during batch digestion of activated sludge: (a) CH_4 yield and (b) CH_4 content.

direct relationship, between RSI dosage and the VS removal rates. This phenomenon is likely due to enhanced methanogenic activity and more efficient conversion of organic matters into methane [1]. A possible explanation for this could be that during microbial iron respiration, iron reducers derive benefit from reduction of Fe(III) by using Fe(III) as the terminal electron acceptor for electrons flowing from organic carbon through the respiratory chain in their metabolism [27]. It is interesting to note that only 0.9% increase in VS reduction was achieved with the RSI dosage increased from 20 to 30 g/L (Fig. 2). A similar phenomenon in CH_4 yield and content of the anaerobic digestion system was observed, as shown in Fig. 1, when the RSI dosage with no significant difference was found between the reactors operated at 20 and 30 g/L. This may be due to iron saturation at the specific solids loading in these reactors. In view of the cost, the best dose of RSI is 20 g/L not 30 g/L for sludge digestion.

3.1.3. The variation of H_2S concentration

As seen in Fig. 3, H_2S concentrations in biogas with different RSI dosages during the anaerobic digestion process were shown to vary. Specifically, for all the digesters, after 4–5 d of operation, the H_2S concentrations in biogas reached peak values, ranging from 7,300 to 14,000 ppmv, and lower H_2S concentrations obtained in the digesters containing RSI. After this initial peak after 5 d of operation, the H_2S concentrations in biogas started to decline in all digesters. As shown in Fig. 3, an obvious decrease of H_2S concentration in biogas could be observed in the digesters with higher dosages of RSI (10, 20 and 30 g/L). By the end of the anaerobic batch digestions, in the presence of RSI concentrations of 1, 5, 10, 20 and 30 g/L, H_2S concentrations with RSI were reduced to 6,869, 4,398, 3,338, 1,992 and 1,887 ppmv, representing 31.3%, 56.0%, 66.6%, 80% and 81.1% less H_2S than that in the control (about 10,000 ppmv), respectively. These results show that RSI addition had a positive effect on both H_2S control in biogas production. The commonly accepted mechanism of electron transfer from RSI to microorganisms is via microbial corrosion and surface oxidation of RSI. RSI was initially observed to be covered by a layer of rust on inner ZVI surface, and this rust consisted of different iron oxides (Fe_2O_3, Fe_3O_4, FeO and FeOOH) [28]. These oxides are understood to react with H^+ (corrosion process), inducing the release of Fe(II) and Fe(III). When Fe(III) enters the liquid phase it is rapidly converted into Fe(II) by iron-reducing bacteria (IRB) [29,30]. Under anaerobic conditions, inner ZVI is oxidized to Fe(II), producing cathodic hydrogen (H_2/[H]) via Eq. (6) [31]. The corrosion process of iron oxide and ZVI exerts a beneficial effect on pH control for the digestion system, and as a consequence, the methane production increases with the applied RSI dosages (Fig. 1). Subsequently, the produced Fe(II) by IRB can precipitate with sulfide by forming FeS (Eq. (7)), which is the proposed mechanism through which RSI addition reduces H_2S concentrations in biogas.

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \quad \Delta G^{\circ\prime} = -5.02 \text{ kJ/mol} \tag{6}$$

$$Fe^{2+} + S^{2-} \rightarrow FeS \tag{7}$$

Superficially, the reactor with 30 g/L had the best performance, with an accumulative methane yield of 210.7 ± 6.5 mL/gVS, a VS reduction of 41.6 ± 2.07, and a H_2S concentration of 1,887 ppmv. However, the acceleration during sludge digestion was negligible compared with dosage of 20 g/L, suggesting that the optimal RSI dose is 20 g/L, and is possible that doses greater than 30 g/L could produce toxic side effects. Manufacturers of combined heat and power (CHP) production units recommend limiting H_2S values to between 0.01 and 0.03% v/v (100–300 ppmv) in order to prevent corrosion in piping systems and equipment [32]. In this study, even under the condition of optimal dosage, H_2S content in biogas far exceeds recommended levels for utilization. However, although biogas sulfide content has to be controlled in order to prevent damage and fulfill the quality standards required in consistence with the chosen application of the biogas, the techniques using RSI described here could reduce the demands necessary to remove residual H_2S present in biogas.

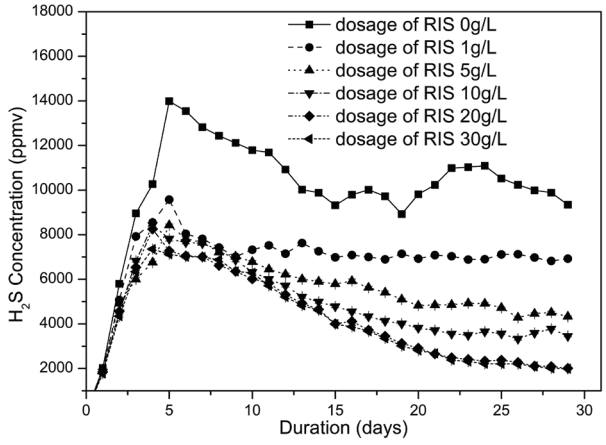

Fig. 3. H_2S concentrations present in biogas from batch digestion of activated sludge in the presence of different RSI dosages (error bars represent standard deviation, $n = 3$).

3.2. Sludge digestion system with RSI amendments during semi-continuous anaerobic/microaerobic digestion

3.2.1. The variation of pH and ORP

To investigate the effects of the limited oxygen supply to the RSI anaerobic system on steady-state performance in this study, the variation of pH and ORP were characterized during semi-continuous digestion experiments (Fig. 4). In P1, which involved neither addition RSI nor supply O_2, the pH decreased from near neutral values to 6.33 over the initial 3 d (Fig. 4). This can be attributed to the build-up of volatile fatty acids (VFAs) during alkaline-pretreated sludge digestion because methanogens grow more slowly than acidogens in the degradative process [33]. Afterwards, pH increased slightly, fluctuating from 6.5 to 6.8 over the remainder of P1. During P2, the RSI (20 g/L) was added into reactor. As expected, the pH in the reactor quickly returned to 7.0 and remained in the near-optimal pH range (6.8–7.2) [34], due to the previously described mechanism of pH regulation through corrosion process. It has been reported [35] that the

point of oxygen supply (headspace or liquid phase) played an important role on hydrogen sulfide oxidation. The H_2S in the biogas was mainly gathered in the headspace of reactors. Thus, the supply of oxygen from the headspace could make the H_2S, O_2 and SOB better blend and contact which was beneficial to desulfurization reaction. Dosage in the headspace could ensure a more stable operation [36]. On the 61st day (period P3), 1 mL pure O_2 was introduced into the reactor headspace, and as shown in Fig. 4, the pH was maintained at ideal levels (6.8–7.2), with similar effects observed at P4, P5 and P6. Supplying limited O_2 could have increased the chemical corrosion of ZVI, as the common consensus is that the principal site of cathodic oxygen reduction (Eq. (8)) and anodic metal dissolution according to Eq. (9) occurs close to RSI edges [37].

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (8)$$

$$Fe(s) \rightarrow Fe^{2+}(aq) + 2e^- \quad (9)$$

$$2H_2O + 4Fe^{2+}(aq) + O_2 \rightarrow 4Fe^{3+}(aq) + 4OH^-(aq) \quad (10)$$

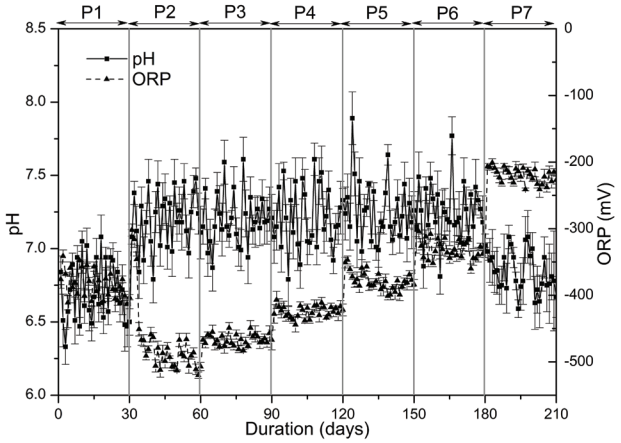

Fig. 4. Variation of pH (■) and ORP (▲) under anaerobic and microaerobic conditions during semi-continuous digestion of activated sludge supplemented with RSI.

When Fe(III) enters liquid phase it will rapidly be converted into Fe(II) by IRB [28,29]. By looking at the fast Fe(III) reduction at the time, two possible reasons could be proposed for the observed pH stabilization: (1) the electrons generated during the degradation of the sludge organics were, to a large extent, utilized for Fe(III) reduction, decreasing the amount of electrons flowing to the intermediates to form fermentation end products, mainly in the form of VFAs; (2) much of the resultant VFAs from the sludge digestion were consumed as the electron donors for Fe(III) reduction. In both the ways, the supplemented RSI acted as an electron sink to alleviate the accumulation of reducing equivalents for VFAs formation, thus preventing the pH from decreasing. However, slight pH drops were observed in P7, mainly fluctuating from 6.6 to 6.9. This can be ascribed to excess oxygen because the system reductive environment was destroyed, which restrained the methanogens activity but did not inhibit acidogenic facultative anaerobes [38].

In the first stage (P1), reactor ORP generally ranged from –410 to –350 mV, which is suitable for anaerobic digestion normal operation. Because of the rapid dissolution of iron oxide and release of ferric ions, the ORP level in P2 was noticeably higher than in P1 in the first 2 d (Fig. 4) and which consequently decreased to a lower lever (about –450 to –500 mV), which should be attributed to the reducing ability of inner ZVI. Previous research indicated that ORP in anaerobic environment can be decreased by as much as 100 mV through addition of ZVI [39]. In P3, micro-oxygenation (1 mL O_2) was started (Table 3), and a slight increase in ORP was observed, fluctuating from –450 to –480 mV. The ORP is a measure of the redox potential and is sensitive to the presence of O_2 in an aqueous solution. In the remaining four stages (P4, P5, P6 and P7), the system ORP increased with stepwise increasing O_2 dosage with fluctuations of –410 to –440 mV, –350 to –400 mV, –300 to –340 mV and –200 to –230 mV, respectively. With respect to P4–P6, although the system ORP levels were increased from –440 to –300 mV, any negative impact on methanogenic activity was non-existent. Instead of decreasing, the average COD removal and average methane yield increased slightly (Table 3). This is consistent with the fact that ZVI created a favorable environment for the growth of methanogens by lowering the ORP (–270 to –370 mV) and increasing the buffer capacity of the system [40]. In comparison, in P7, excess oxygen disrupted the

Table 3
Reactor performance during semi-continuous anaerobic and microaerobic digestion of activated sludge supplemented with rusty scrap iron (RSI) over seven periods of operation associated with stepwise increases in oxygen concentration

	P1	P2	P3	P4	P5	P6	P7
COD in (mg/L)	31,253	31,253	31,253	31,253	31,253	31,253	31,253
Average COD out (mg/L)	19,314	13,314	13,658	14,314	13,908	14,126	17,189
Average COD removal (%)	38.2	57.4	56.3	54.2	55.5	54.8	45.0
Average methane yield (mL/gCOD)	218.76	306.00	300.59	298.54	298.90	301.13	261.39
Average H_2S concentration (ppmv)	12,504	1,933	776	484	234	75	68
Average O_2 concentration (ppmv)	–[a]	–	158	759	1,230	2,083	8,438
Average CH_4 concentration (ppmv)	557,964	656,972	642,738	634,655	630,789	648,816	614,308

[a]Undetectable.

system reductive environment that is necessary for methanogens growth, followed by the reduction of average COD removal and average methane yield (Table 3).

3.2.2. The anaerobic/microaerobic digestion performance

The variation of daily COD removal and daily methane yield for entire duration of the semi-continuous digestion is illustrated in Fig. 5. In this study, the methane yield is the ratio of volume of methane produced to COD of treated wastewater. The variation trend of COD removal is similar to methane yield. It is possible that methane production is the predominant way of COD removal in anaerobic digestion systems with low hydrogen production. The COD removal and methane yield in P1 (neither RSI supplementation nor O_2 supply), which were considered data for the baseline period, were 38.2% and 218.75 mL/g COD on average (Table 3). After RSI supplementation in P2 COD removal and CH_4 yield increased noticeably in the initial 4 d, with subsequent fluctuation within a narrow range. A 1.50-fold increase in average COD removal and a 1.41-fold increase in average methane yield were achieved compared with P1. This is attributed to the reduction of Fe(III) oxides on the RSI surface, which promoted microbial hydrolysis–acidification of complex matter, providing more organic matter for methanogenesis [21]. In addition, a previous study has suggested that RSI could enhance decomposition of propionate and created further improvements on the propionate conversion [21]. While propionate could not be utilized directly by methanogens, its biotransformation products could, ultimately enhancing methane yield. In P3–P6 (i.e., stepwise increase in oxygen concentration), the COD removal and the methane yield were almost equal to that of P2 (Fig. 5), which fluctuated in small scope arising from the variability of feeding composition. The COD removal during P2, P3, P4, P5 and P6 was on average 57.4%, 56.3%, 54.2%, 55.5% and 54.8%, respectively. And P2–P6 achieved average methane yields of 306.00, 300.59, 298.54, 298.90 and 301.13 mL/g COD, respectively. In P7, the COD removal and methane yield declined simultaneously due to system reductive environment destruction (Fig. 4). As noted above, these results are consistent with those reported in literature, even on an industrial scale, it has been broadly demonstrated that the presence of limited amounts (not exceeding 15 mL in this study) of O_2 in digesters does not negatively affect digestion performance [41,42].

3.2.3. H_2S and O_2 concentration in biogas

The H_2S content in biogas produced in P1 (under anaerobic conditions) had a larger fluctuation range (Fig. 6) and was on average 12,504 ppmv (Table 2). In P2, RSI supplementation could drastically reduce the H_2S content in biogas to achieve an average of 1,933 ppmv (see illustration of previous section), representing an overwhelming 84.5% reduction compared with P1. Microaerobic conditions were applied in the following five periods (P3–P7) and gradually the O_2 concentration was increased stepwise (Table 2). The result showed that the content of H_2S in biogas was decreased with increasing of O_2 dose, and, but naturally the O_2 content in the resulting biogas was higher. Specifically, from P3 to P7, the average H_2S concentration in biogas was 776, 484, 234, 75 and 68 ppmv, respectively, while O_2 concentrations were on average 158, 759, 1,230, 2,083 and 8,438 ppmv, respectively. It is worth noting that at even the highest micro-oxygenation level (P7), the surplus O_2 content greatly increased by 305.1% compared with P6, while the biogas H_2S content was almost equal to that in P6, indicating diminishing returns past this concentration. The considerable increase in biogas O_2 concentration also accompanied by a decrease of methane production in P7 and decreased O_2 consumption. Regarding the biogas H_2S content, it hardly changed in comparison with P6, which could be related to two possibilities: (1) the biogas desulfurization took place predominantly in the gas–liquid interface [24]. Although sufficient O_2 was supplied, it is hard to accommodate excessive SOB in this limited space due to the unavailability of H_2S; (2) the precision of the gas analyzer to detect H_2S could also be limited at lower values. In P6, the average methane yield increased by 37.65% comparing with P1. What's more, the H_2S removal efficiency in biogas decreased by 99.40% in this case, and a concentration below 100 ppmv was reached,

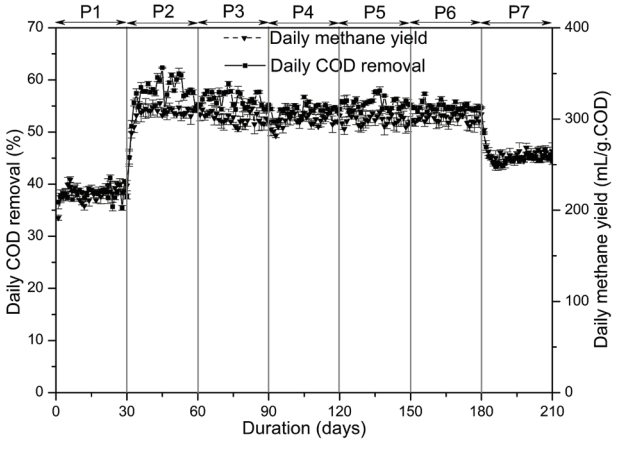

Fig. 5. Daily COD removal (■) and daily methane yield (▼) under anaerobic and microaerobic conditions during the digestion of sludge.

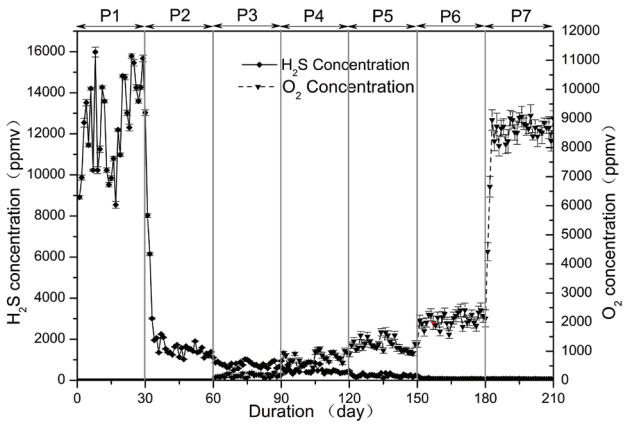

Fig. 6. Hydrogen sulfide (♦) and oxygen (▼) concentrations under anaerobic and microaerobic conditions during semi-continuous digestion of activated sludge supplemented with RSI.

guaranteeing levels adequate for CHP (100–300 ppmv), which represents a major use of biogas, thus avoiding high costs associated with desulfurization.

3.3. Sulfur balance

Microaeration had significant and rapid effect on sulfur distribution in reactor, as did RSI supplementation. All sulfur species (i.e., H_2S, S^{2-}, HS^-, S^0, $S_2O_3^{2-}$ and SO_4^{2-}) exist in three states: solid, liquid and gas. The mass of all relevant species were measured and are summarized in Table S1, while Fig. 7 shows species distribution before and after the experiment. Before anaerobic/microaerobic digestion, the sulfur species in the digester included organic sulfur-containing compounds in the waste sludge and added sulfate. The dissolved sulfur compounds obtained from the waste sludge were mainly sulfate and sulfide, while the thiosulfate was negligible. After anaerobic/microaerobic digestion, six major streams of sulfur species in the digester were identified: (1) sulfur compounds in waste sludge, (2) iron sulfide precipitation, (3) elemental sulfur S^0 (deposition in the headspace of the reactors), (4) sulfide in liquid phase, (5) sulfide oxidation products ($S_2O_3^{2-}$ and SO_4^{2-}) and (6) sulfur in biogas (as hydrogen sulfide).

The solid form of sulfur in this system is mainly consisted of sulfur compounds in waste sludge, iron sulfide precipitation and elemental sulfur S^0 (deposition in the headspace of the reactors). It can be seen that after anaerobic/microaerobic digestion, the amount of sulfur compounds in sludge decreased across all the periods, which resulted from the biological reduction of both sulfate and organic sulfur compounds by SRB. In P1, sulfur compounds in sludge decreased by 25.29%, while percentage reduction of more than 40% was achieved in the later periods. Combining RSI with O_2 can enhance cell lysis, which released more organic sulfur compounds which can be reduced to sulfide by SRB [25,43,44]. Only 1.44% of the sulfur dosed was present in the solid phase of the iron sulfide precipitation in P1, while over 26% was in P2 due to the addition of RSI. Starting from P3, supply of micro-oxygen promoted the generation of Fe(II) and Fe(III), which further increased the mass of iron sulfide precipitate, which ranged from 45 to 60 mg. In addition, oxygen can also directly to react with sulfide as an electron acceptor. Furthermore, sulfide was transformed into elemental sulfur through SOB activity as an electron donor. In P3 through P6, 26.94%, 32.70%, 37.24% and 42.39% of the sulfur dosed was transformed into elemental sulfur, respectively, while in P7 a 30.07% reduction was achieved (Table S1). The possible reason could be that excess oxygen was supplied, leading to part of sulfide being oxidized to thiosulfate or sulfate [45].

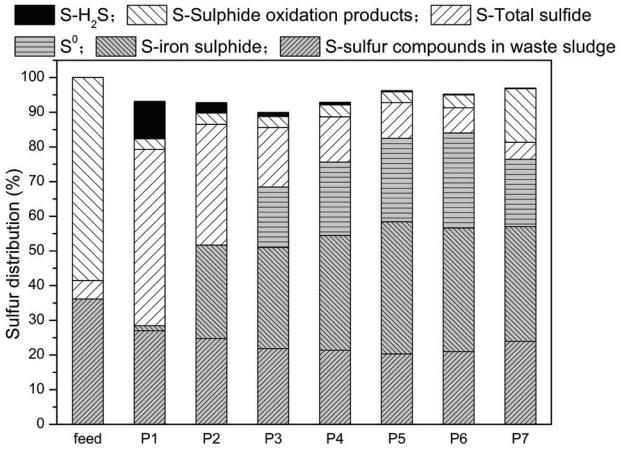

Fig. 7. Calculated sulfur balance during semi-continuous anaerobic and microaerobic digestion of activated sludge supplemented with rusty scrap iron (RSI) over seven periods of operation associated with stepwise increases in oxygen concentration: (1) sulfur compounds in waste sludge, (2) iron sulfide precipitation, (3) elemental sulfur S^0, (4) sulfide in liquid phase, (5) sulfide oxidation products and (6) sulfur in biogas.

The dissolved forms of sulfur in this system are mainly sulfide (S^{2-}, HS^- and H_2S) and sulfide oxidation products ($S_2O_3^{2-}$ and SO_4^{2-}). The sulfide detected in the liquid from P1 to P7 accounted for 50.82%, 34.81%, 17.18%, 13.02%, 10.30%, 7.25% and 4.89% of the sulfur dosed, respectively. Thus, the addition of RSI into the reactor decreased not only the concentration of hydrogen sulfide in biogas, but also the concentration of sulfide in the liquid, and combining limited amounts of O_2 with RSI achieved this more effectively. During the first six periods, only negligible amounts (stabilized at around 5 mg) of sulfide oxidation products ($S_2O_3^{2-}$ and SO_4^{2-}), were found in the liquid. Díaz et al. [46] discovered that sulfate was found to be easily accessible for sulfate-reducing microorganisms, while organic sulfur in the form of proteins, or cell constituents, was only partly reduced, and a large portion left the bioreactor unchanged. However in P7, the amounts of sulfide oxidation products significantly improved to 23.97 mg. Indeed, the optimum ORP for CH_4 reducing bacteria is below –230 mV while an ORP value above –280 mV is inhibitory to SRB [47]. In P7, the reactor ORP already exceeded the value (Fig. 4).

The gaseous form of sulfur in this system was mainly H_2S. The digester was fed with 154.69 mg/d of sulfur, 10.82% was found in biogas during P1, while only 2.99% of sulfur was detected in biogas during P2 resulting from addition of RSI ($Fe^{2+} + S^{2-} \rightarrow FeS$). Starting from P3, O_2 was added into the reactor to enhance the biogas desulfurization. A fraction of O_2 acting electron acceptor directly reacting with sulfides, and another fraction of O_2 corroded iron producing Fe(II) and Fe(III), which then also reacted with sulfide. Of all sulfur dosed into reactor, 2.99%, 1.18%, 0.71%, 0.36%, 0.21% and 0.11% was found in biogas, respectively, for P3, P4, P5, P6 and P7 (Table S1).

The outputs of sulfur accounted for 93.12%, 92.76%, 89.95%, 92.84%, 96.21%, 95.19% and 96.93% of inputs (Table S1), respectively. This 'lost' sulfur, comparing inputs and outputs, was not analyzed in this research. There are several possible explanations for this lack of sulfur in the balance. First, sulfur emission in the biogas may not be limited to H_2S but other possible S-containing gaseous forms emitted in the biogas but were not taken into account in our balance. These include dimethyl sulfide, carbon disulfide, mercaptans, etc. Second, total sulfur determination may have been biased by the high total dissolved sulfide content, which could have volatilized during the drying procedure before elemental analysis [48].

4. Conclusion

Combining microaerobic desulfurization with RSI amendment during WAS digestion was investigated to develop a cost-effective and environment-friendly technology for H_2S removal and energy recovery. The optimal condition is combining a dosage of 20 g/L RSI with supply of 15 mL O_2 (9.43 NL oxygen/Nm^3 biogas) to the digestion system. In this case, the negative influence of micro-oxygen on digestion performance was found to be negligible. An average methane yield of 301.13 mL/gCOD was reached and biogas H_2S content remained steadily below 100 ppmv. Under optimal dosing conditions, H_2S content in biogas could fulfill the quality standards required for subsequent application of the produced biogas and obviating the need for desulfurization processes.

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Supplementary material

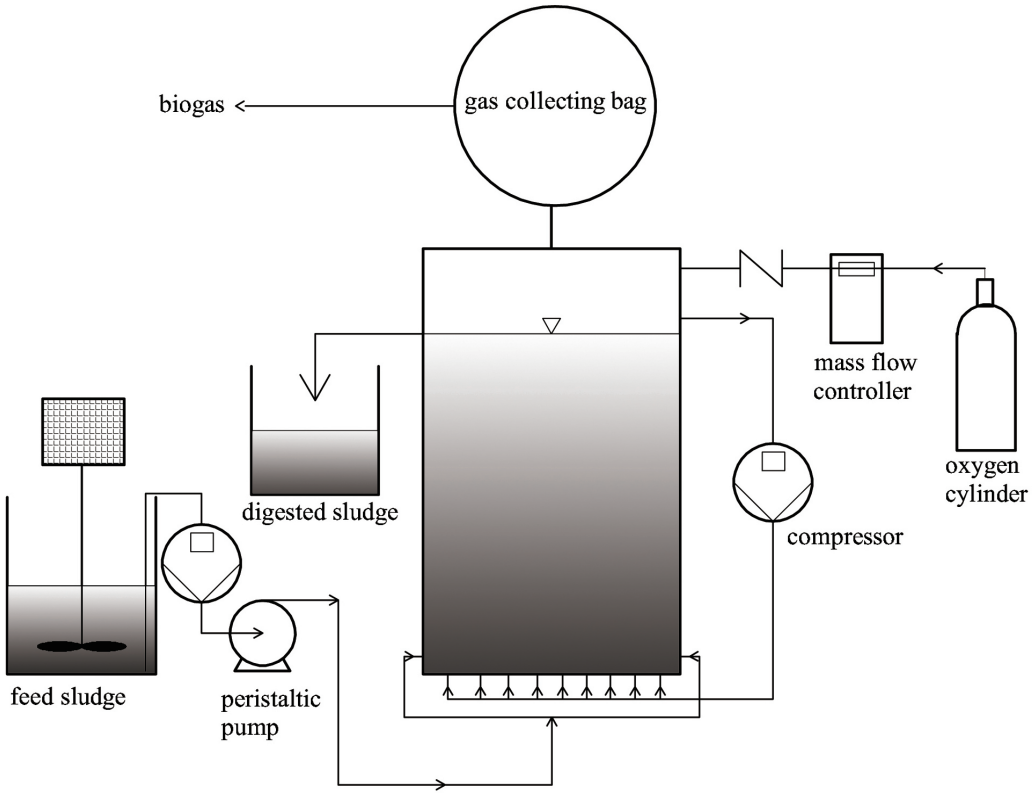

Fig. S1. Lab-scale bioreactor diagram for semi-continuous anaerobic and microaerobic digestion of activated sludge supplemented with rusty scrap iron (RSI).

Table S1
The mass distribution of sulfur compounds (mg) in the system before and after semi-continuous anaerobic and microaerobic digestion of activated sludge supplemented with rusty scrap iron (RSI) over seven periods of operation associated with stepwise increases in oxygen concentration

	Sulfur species	Feed	P1	P2	P3	P4	P5	P6	P7
S in solid phase (mg)	S-sulfur compounds in waste sludge	55.92	41.77	38.26	33.82	33.05	31.40	32.47	37.09
	S-iron sulfide	–[a]	2.23	41.67	45.09	51.22	58.91	55.11	51.08
	S^0	–	–	–	26.94	32.70	37.24	42.39	30.07
S in liquid phase (mg)	S-total sulfide (S^{2-}, HS^- and H_2S)	8.26	78.62	53.84	26.57	20.15	15.93	11.21	7.56
	S-sulfide oxidation products ($S_2O_3^{2-}$ and SO_4^{2-})	90.51	4.7	5.1	4.9	5.4	4.8	5.74	23.97
S in gaseous phase (mg)	S-H_2S	–	16.73	4.62	1.82	1.10	0.55	0.33	0.17
Total elemental sulfur (mg)[b]		154.69	144.05	143.49	139.14	143.62	148.83	147.25	149.94

[a]Not detected.
[b]Total elemental sulfur: total sulfur as sum of S in all sulfur compounds.