Continuous Sulfidogenic Wastewater Treatment with Iron Sulfide Sludge Oxidation and Recycle

Dongyang Deng, Lian-Shin Lin *

Civil and Environmental Engineering

West Virginia University

Morgantown, WV 26506-6103

*Corresponding author: lianshin.lin@mail.wvu.edu

Abstract

This study evaluated the technical feasibility of packed-bed sulfidogenic bioreactors dosed with ferrous chloride for continuous wastewater treatment over a 450-day period. In phase I, the bioreactors were operated under different combinations of carbon, iron, and sulfate mass loads without sludge recycling to identify optimal treatment conditions. A COD/sulfate mass ratio of 2 and a Fe/S molar ratio of 1 yielded the best treatment performance with COD oxidation rate of 786 ± 82 mg/(L·d), which resulted in $84 \pm 9\%$ COD removal, $94 \pm 6\%$ sulfate reduction, and good iron retention ($99 \pm 1\%$) under favorable pH conditions (6.2-7.0). In phase II, the bioreactors were operated under this chemical load combination over a 62-day period, during which 7 events of sludge collection, oxidation, and recycling were performed. The collected sludge materials contained both inorganic and organic matter with FeS and FeS₂ as the main inorganic constituents. In each event, the sludge materials were oxidized in an oxidizing basin before recycling to mix with the wastewater influent. Sludge recycling yielded enhanced COD removal ($90 \pm 6\%$ vs. $75 \pm 7\%$), and better effluent quality in terms of pH (6.8 ± 0.1 vs. 6.5 ± 0.2), iron (0.7

± 0.5 vs. 1.9 ± 1.7 mg/L), and sulfide-S (0.3 ± 0.1 vs. 0.4 ± 0.1 mg/L) removal compared to the baseline operation without sludge recycling during phase II. This process exhibited treatment stability with reasonable variations, and fairly consistent sludge content over long periods of operation under a range of COD/sulfate and Fe/S ratios without sludge recycling. The bioreactors were found to absorb recycling-induced changes efficiently without causing elevated suspended solids in the effluents.

Keywords

COD/sulfate; Fe/S; sulfidogenic; wastewater treatment; sludge recycle; COD oxidation rate

1. Introduction

Introducing acid mine drainage (AMD) in municipal wastewater (MWW) can offer multiple environmental and energy benefits over conventional methods such as activated sludge (Deng and Lin 2013, McCullough et al. 2008, Strosnider et al. 2011a, Strosnider et al. 2011b, Winfrey et al. 2010). In such co-treatment systems, sulfate reducing bacteria (SRB) are used to facilitate organic oxidation while sulfate is reduced to (bi)sulfide under anaerobic conditions. Such treatment is energy efficient because it does not require aeration for microbial oxidation of organics in the wastewater. Additional benefits include reduced CO₂ production and emission, removal of selected heavy metals (e.g., Cd, Cu, Hg, Pb and Zn) due to their low solubility with sulfide (Barnes et al. 1991, Bhattacharyya et al. 1981, Jong and Parry 2003, Strosnider et al. 2013), and low biological yield (Hoehler et al. 2001, Postgate 1979).

In sulfidogenic treatment, the degree of organics removal depends on the relative amount of sulfate, and the chemical oxygen demand (COD) to sulfate ratio was typically used to examine such effects (Damianovic and Foresti 2007, Friedl et al. 2009, Jeong et al. 2008, Lens et al. 1998, Vossoughi et al. 2003). Theoretically, enough sulfate is available to completely oxidize the organics when the COD/sulfate ratio is above 0.67 (Rinzema and Lettinga 1988). In reality, optimum COD/sulfate ratio is influenced by the composition and concentration of organic matters. Active competition was observed between SRB and methane-producing bacteria (MPB) when the COD/sulfate ratio was 1.7-2.7, and SRB were dominant over MPB when the COD/sulfate ratio was greater than 1.7 (Choi and Rim 1991).

One potential issue with sulfidogenic treatment of MWW where AMD does not co-exist is an insufficient amount of sulfate for the oxidation of organics given the typical sulfate and organics concentrations of MWW (Metcalf et al. 2013). Incorporating iron in the sulfidogenic treatment can overcome this potential drawback and enable multiple (bio)chemical reactions in engineering designs that facilitate removal of a wide range of contaminants from MWW. Specifically, low solubility of iron phosphate can be an effective mechanism for retaining phosphorus from wastewater and reducing nutrient loads to receiving waters. Precipitation of iron sulfide due to its low solubility (amorphous ferrous sulfide $K_{sp} \approx 10^{-3.05}$, (Emerson et al. 1983) can limit sulfide toxicity on SRB and control sulfide levels in the treated effluents. The formed iron sulfide sludge materials can be oxidized into ferric sulfate and recycled to the wastewater influent to supplement sulfate and iron for continuous treatment. With the abundance and widespread presence of iron, tremendous opportunities exist for incorporating iron in

innovative MWW treatment technologies to realize the ‘green’ benefits of this treatment approach.

The goal of this study was to evaluate the technical feasibility of an innovative iron-dosed sulfidogenic treatment process with iron sulfide sludge recycle to supplement sulfate and iron for continuous wastewater treatment. A bench-scale treatment process containing two packed-bed bioreactors was constructed to treat synthetic wastewater under a range of the key chemical factors (i.e., COD/sulfate ratio and Fe/S ratio) to optimize treatment performance. The treatment performance of the process and its potential were evaluated by COD removal efficiency and effluent quality. Physicochemical properties of the sludge materials were characterized and the effects of recycling were assessed by contrasting the treatment performance parameters between treatment periods with and without sludge recycling. Chemical states and mass balance of key elements, Fe and S, at different stages of the treatment process were examined to characterize the treatment process and illustrate the treatment mechanisms. Solid content and composition were monitored over long-term operation of the bioreactors.

2. Material and Methods

2.1 Bench-scale sulfidogenic treatment process

A bench-scale treatment process consisting of a wastewater reservoir, ferrous chloride reservoir, two parallel sulfidogenic bioreactors, and an oxidizing basin was constructed and used in this study (Figure 1).

2.1.1 Wastewater reservoir

A 57-L tank was used as a wastewater reservoir to supply wastewater for continuous sulfidogenic treatment. A synthetic wastewater containing 2.26 mM ethanol ($\text{C}_2\text{H}_6\text{O}$), 0.45 mM lactose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}$) and 1.61 mM sodium acetate ($\text{C}_2\text{H}_3\text{O}_2\text{Na}\cdot 3\text{H}_2\text{O}$), 1.68 mM sodium bicarbonate (NaHCO_3), and trace elements (Supplementary material Table S1, 5 ml/L influent) (Diekert et al. 1992) was used in this study. In addition, different amounts of sodium sulfate (0.56-4.44 mM Na_2SO_4) were mixed with the synthetic wastewater to allow testing of the effects of COD/sulfate ratio on the sulfidogenic treatment.

2.1.2 Ferrous chloride reservoir

A 4-L tank containing a ferrous chloride solution ($\text{FeCl}_2\cdot 4\text{H}_2\text{O}$, pH = 3.2 – 3.4) was used to evaluate the effects of iron dose on the sulfidogenic treatment of the wastewater. A range of ferrous chloride concentrations (0.56-17.76 mM) resulting in a range of Fe/S molar ratios (1 – 4) was used to investigate the effects of ferrous dosing.

2.1.3 Sulfidogenic bioreactors

Duplicate sulfidogenic bioreactors (BR1 and BR2, 2.5 L each) were constructed and used to treat the synthetic wastewater. The bioreactors were made of an acrylic cylinder with inlets on the top for the synthetic wastewater and ferrous dosing. Each bioreactor was packed with plastic media (Kaldnes K1, specific surface area = $500 \text{ m}^2/\text{m}^3$, Evolution Aqua Ltd, UK) for attached growth of microorganisms. A perforated acrylic plate was used to support the packing media and a coned-shaped bottom was used to allow sludge settling and collection. There were ports on the

side of the reactors for internal recirculation to promote hydraulic mixing and treatment efficiency. The attached growth design allowed development of microbial communities more resistant to potential toxic effects of metals (Parkin and Speece 1983) and convenient separation of iron sulfide precipitates from the attached biomass by their settling to the cone-shaped bottom of the bioreactors. The bioreactors each provided a working volume of approximately 1.5 L with the packing media and sludge biomass.

Anaerobic digester sludge from a municipal wastewater treatment plant (Star City, West Virginia) and AMD sludge from a mine portal along Dunkard Creek (near Bobtown, Pennsylvania) were collected, mixed at 1:1 volume ratio and used to inoculate the bioreactors. No additional growth media was used during the inoculation. The bioreactors were operated at room temperature (21 ± 1 °C) and under anaerobic condition. The air tight bioreactors were sparged with syringe filtered (0.45 µm, Fisherbrand, Ireland) N₂ gas prior to operation.

2.1.4 Oxidizing basin

The oxidizing basin was a 4L wide-mouth conical flask with 30 pieces of plastic media (Kaldnes K1, Evolution Aqua Ltd, UK) for attached growth of microorganisms. During sludge oxidation operation (6 days each time), sludge samples from the bioreactors were collected and added to the oxidizing basin daily. A magnetic stirrer was used to mix the sludge under aerobic conditions (i.e., open flask with mixing) to transform iron sulfide minerals into ferric and sulfate ions. At the end of the oxidation period, the oxidized sludge solution was then mixed with the wastewater influent (1:1 volume ratio) to evaluate the effects of sludge recycling. Samples of the sludge were taken for chemical analyses before and after mixing with the wastewater.

2.2 Experimental design

The bench-scale experiments were conducted in two phases. In phase I (450-day study period), the synthetic wastewater was continuously treated in the bioreactors to determine optimal conditions of COD/sulfate ratio and ferrous loading rate. In this phase, the experiments were conducted without sludge oxidation or recycling, and both BR1 and BR2 were operated under the same conditions as duplicates. The bioreactors were tested for their performance under a range of the COD/sulfate mass ratios (1, 1.3, 2, 4, and 8) and Fe/S molar ratios (1, 2 and 4) with each ratio combination lasting for approximately 2 months (1-month acclimation followed by a 1-month sampling period). In these experiments, influent flow rate (wastewater plus $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 3.5 L/d) and an internal recirculation (18.4 L/d, flow ratio = 5.3) were used. During each experiment, influent and effluent samples (20 each) were collected to quantify COD oxidation and sulfate reduction in the bioreactors. Iron retention, defined as loss of iron mass to chemical precipitation in the sludge or retained in the bioreactors, was calculated by the difference of the iron load between the influent and the effluent normalized by the influent load (%). The phase I results were analyzed to select the optimal chemical loading of COD/sulfate and ferrous iron for phase II experiments.

In phase II, the bioreactors were operated with periodic iron sulfide sludge collections and recycling over a 62-day period. Both BR1 and BR2 were operated under the same chemical loads. In each event, sludge materials (100 mL wet sludge from each bioreactor) were extracted from the bottom of the bioreactors daily and added to the oxidizing basin daily for six days. On day 6, the oxidized sludge solution was recycled to the bioreactors at the same flow rate as the

synthetic wastewater. The averaged retention time for sludge oxidation was 3 days and the recycling of the oxidized solution lasted approximately 9 hours each time. During the 62-day operation, 7 occasions of sludge recycling were performed. Regular samplings were done to allow comparisons of the treatment performance with and without the sludge recycling.

2.3 Control experiments

Control experiments were also conducted to quantify the contribution of abiotic processes to COD removal and sulfate reduction. The mixed wastewater and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ solutions were sonicated (10 min) and then transferred into an anaerobic reactor which was maintained under same conditions as the sulfidogenic bioreactors. Microbial activities were inhibited by adding a 2 % sodium azide solution. The control experiments lasted for 30 days, and samples were taken every 2 days. The samples from the control experiments were analyzed using the same methods as the sulfidogenic treatment samples.

2.4 Chemical Analyses

An YSI meter with pre-calibrated probes (YSI 63) was used to measure pH and conductivity. Autotitrators were used for alkalinity (Thermo Scientific Orion 950, Standard Method 2320 B) and acidity (Mettler Toledo DL50, Standard Method 2310 B) analyses. Samples for iron analyses were preserved with concentrated trace metal-grade nitric acid and stored at 4 °C until nitric acid-perchloric acid digestion using an atomic adsorption spectroscopy (AAS,

Perkin Elmer 3100) following Standard Method 3030H. The digested samples were filtered through 0.45- μ m nylon filters before the analysis for total iron concentration. Ferrous iron was determined using the 1,10 Phenanthroline method (Standard Method 3500 B) and ferric iron was determined by the differences between total and ferrous iron concentrations (Standard Method 3500 B).

Total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), and volatile suspended solids (VSS) were determined following the standard methods (APHA 2005). Sulfate concentrations were measured using a turbidimetric method (USEPA method 375.4) with a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S). COD concentrations were determined using a closed reflux, colorimetric method with a spectrophotometer (ASTM D1252-06 2006), and sulfide concentrations measured by a methylene blue method (USEPA method 376.2). Duplicates of sulfate and COD concentrations were measured for each sample. Redox potential (ORP) was measured using a polished platinum probe with an Ag/AgCl electrode as a reference (EW-27018-40, Cole Parmer, Vernon Hills, IL, USA).

2.5 Sludge characterization

The sludge materials extracted from the bioreactors were analyzed for solid contents including TSS, TDS, VSS, and NVSS. They were also analyzed by X-ray photoelectron spectroscopy (XPS, Physical Electronics PHI 5000 VersaProbe) to determine the chemical states of iron and sulfur. The sludge samples were first dried in a closed chamber filled with calcium sulfate and flushed with nitrogen to remove the moisture content and prevent sludge oxidation

(Karamalidis et al. 2008) until the weight was constant. The sludge samples were then powdered and the sample powder was mounted in the standard sample holder with a zero reflective quartz plate (MTI Corporation, CA) placed underneath. XPS spectra were obtained using XPS equipped with a monochromatized Al K α X-ray source (1487 eV). The base pressure in the analytical chamber was 10^{-7} Pa. The instrument work function was set to give a value of 84 eV for the Au line of metallic gold. Narrow region photoelectron spectra were acquired to obtain chemical state information for iron and sulfur.

Surface morphology and elemental composition were analyzed using a scanning electron microscope (SEM, Hitachi S-4700F) coupled with energy dispersive X-ray spectroscopy (EDX, EDAX Genesis). The sludge samples were prepared using the same method as XPS (dried in the closed chamber) and analyzed under an accelerating potential of 5-10 kV. Qualitative elemental analysis of the sludge samples was conducted by EDX spectrometry operated under an accelerating potential of 20 kV. All samples were mounted on aluminum sample stubs and gold-coated to minimize surface charging.

3. Results and Discussion

3.1 Phase I: Sulfidogenic treatment without sludge recycle

3.1.1 Control vs. sulfidogenic treatment

In the control experiments, 10-25% COD removal was observed. This was substantially less than that observed in the sulfidogenic bioreactors. Sulfate reductions were negligible

(Figures 2a and 2b). This indicates that COD removal in the sulfidogenic bioreactors was predominantly biotic due to SRB.

3.1.2 Steady state evaluation

A transient study during the 1-month acclimation period showed that the bioreactors reached a steady state by day 20 (Supplementary material, Figure S1). This suggested that the 1-month period followed by a 1-month sampling period was sufficient for studying the effects of different COD/sulfate and Fe/S ratios.

3.1.3 Effects of COD/sulfate and Fe/S ratios

Over the range of COD/sulfate ratios tested (i.e., 1 – 8), COD removal (%) decreased with the ratio (Figure 2a) reflecting the decreasing availability of sulfate as the electron acceptor for COD removal. Sulfate reduction (%) exhibited the opposite trend (Figure 2b). Under each COD/sulfate ratio, the bioreactors were progressively less efficient at COD oxidation and sulfate reduction as Fe/S molar ratio increased from 1 to 4. A stoichiometric ratio of 1:1 (Fe/S) yielded the most favorable treatment performance for both COD oxidation and sulfate reduction. The decreased treatment performance was mainly due to the effect of low pH on SRB from the increased loading of acidic ferrous chloride solution (Figure 2d). The optimal pH for SRB is 5.8 - 8.0 (Al Zuhair et al. 2008, Reis et al. 1992, Vogels et al. 1988, Widdel 1988). In addition to the pH effects, the excessive loads of ferrous ion over sulfate resulted in substantially higher levels of dissolved iron in the bioreactor effluent (Figure 2c).

Of the different combinations of the chemical ratios, a COD/sulfate of 2 (mass ratio) and Fe/S of 1 (molar ratio) yielded the best treatment performance with 84 ± 9 % COD removal, 94 ± 6 % sulfate reduction, and good iron retention ($99 \pm 1\%$) under favorable pH conditions (6.2-7.0). This optimal COD/sulfate ratio was consistent with previous sulfidogenic studies (Choi and Rim 1991, Damianovic and Foresti 2007, El Bayoumy et al. 1999, Hirasawa et al. 2008, Velasco et al. 2008).

3.1.4 COD oxidation rate

The COD oxidation rate was calculated based on the mass balance equation under steady-state conditions (Supplementary material). The average reaction rate ranged from 338-865 mg/L·d under different chemical load combinations (Figure 3a). These rates were comparable or better than the maximum reaction rates (v_{max}) reported in other sulfidogenic studies using mixed cultures and in general lower than those using pure SRB cultures (Table 1). Sulfate reduction rates were also calculated and the average rates ranged from 105-726 mg/(L·d) under different chemical load combinations (Figure 3b). These reactions rates compared favorably to a co-treatment system used to treat a high-strength AMD and municipal wastewater (54 mg/(L·d), Strosnider et al. 2011a) and a passive SRB bioreactor under optimum field conditions (29 mg/(L·d), URS 2003). The sulfate reduction rates in the current showed a wider range than those observed in a laboratory study using ethanol, lactic acid and glycerol as electron donors (250-300 mg/(L·d), Kolmert and Johnson 2001).

Table 1 Michaelis-Menten kinetic parameters for COD oxidation by sulfate reducing cultures

Culture	v_{max}	K_m	Reference
---------	-----------	-------	-----------

	mg/(L·d)	mg/L	
Enriched culture from anaerobic digester sludge	475.2	4.3	Deng et al. (2016)
Enriched culture from mining areas	316.8	--	Sahinkaya et al. (2007)
Mixed culture of SRB and methanogens	47.5-50.4	2.7-3.5	Kaksonen et al. (2003)
Mixed culture of SRB and methanogens	936	9.5	Yoda et al. (1987)
<i>Desulfobacter postgatei</i>	576-1728	13.6	Schönheit et al. (1982)
<i>Desulfobacter postgatei</i>	4320-4464	3.8–4.5	Ingvorsen et al. (1984)
<i>Desulfobacter postgatei</i>	10224-13536	NR	Widdel (1988)
<i>Desulforhabdus amnigenus</i>	2448	35	Oude Elferink (1998)
<i>Desulfobacca acetoxidans</i>	3600	35	Oude Elferink (1998)

3.1.5 Sludge morphology and chemical composition

SEM-EDX analysis indicated significant presence of iron and sulfur in the biogenic sludge (Supplementary material, Figure S2). XPS analysis on the biogenic sludge material showed the presence of sulfide ion (161.4 and 162.9 eV) and significantly strong presence of ferrous iron (708.6 eV) (Supplementary material, Figure S3). The analysis revealed atomic percentages of C: O: Fe: S as 41:30:12:17, which corresponds to weight percentages of 22:22:31:25. These results suggest the co-existence of ferrous sulfide (FeS) and pyrite (FeS₂) as a result of the sulfidogenic treatment.

3.2 Phase II: Sulfidogenic treatment with iron sulfide sludge oxidation and recycle

Based on the Phase 1 results, a COD/sulfate mass ratio of 2 and a Fe/S molar ratio of 1 (i.e., COD = 1384 mg/day, SO₄²⁻ = 692 mg/day, and Fe²⁺ = 404 mg/day) were selected to evaluate the technical feasibility of the sulfidogenic treatment with sludge recycling. Effects of the sludge oxidation and recycling are described and discussed in the following sections.

3.2.1 Sludge oxidation

In the oxidizing basin, the mixed influent pH ranged from 5.9 - 6.2 after the oxidized sludge solution ($\text{pH } 2.3 \pm 0.02$) was mixed with wastewater influent. The relatively lower pH in the oxidizing basin compared to the synthetic wastewater and those in the bioreactors (6.2 ± 0.4), was a result of FeS/FeS_2 oxidization, which generated acidity (Schippers and Jørgensen 2002).

3.2.2 COD removal, sulfate reduction, and pH

Recycling of the oxidized sludge materials caused obvious effects on all relevant water quality parameters (Figure 4). Compared to baseline operation, sludge recycling caused significant increases in influent COD (653 ± 87 mg/L vs. 395 ± 22 mg/L) and sulfate-S (94 ± 12 vs. 71 ± 11 mg/L, respectively) concentrations (Figures 4a, b). The changes in chemical loads due to sludge recycling resulted in enhanced COD removal ($90 \pm 6\%$ vs. $75 \pm 7\%$), but similar sulfate reduction efficiency ($92 \pm 4\%$ vs. $93 \pm 7\%$) as evident in the overall lower effluent COD (65 ± 33 mg/L vs. 100 ± 27 mg/L) and sulfate-S concentrations (7.0 ± 3.4 mg/L vs. 5.0 ± 5.1 mg/L) (Figures 4a, b). The sludge recycling also caused slight decreases in influent pH (7.3 ± 0.2 vs. 7.7 ± 0.3 , Figure 4c). This did not negatively impact SRB, and resulted in an elevated pH in the effluent (6.8 ± 0.1 vs. 6.5 ± 0.2) due to additional alkalinity generation from increased sulfate reduction. Overall, sludge recycling yielded enhanced COD removal even with the additional COD loads from the recycling, and resulted in better effluent quality in terms of COD and pH.

3.2.3 TDS, TSS and VSS

Sludge recycling resulted in elevated concentrations of influent TSS (884 ± 96 vs. 16 ± 5 mg/L) and VSS concentrations (493 ± 41 vs. 11 ± 5 mg/L), and lower TDS concentrations (756 ± 100 vs. 907 ± 60 mg/L) (Figures 4d, e, f). The bioreactors were found to absorb the changes in these material loads efficiently judging from the slightly elevated TSS (34 ± 33 vs. 23 ± 17 mg/L), VSS (24 ± 20 vs. 14 ± 12 mg/L) and slightly higher TDS (917 ± 190 vs. 766 ± 95 mg/L) in the effluents.

3.2.4 Iron and sulfur

The sludge recycling also supplemented iron to the synthetic wastewater and resulted in increased total iron concentrations in the influent to the bioreactors (161.4 ± 8.8 vs. 118.0 ± 5.2 mg/L, Figure 4g). The additional iron loads subsequently lowered the sulfide-S levels in the effluents (0.3 ± 0.1 vs. 0.4 ± 0.1 mg/L, Figure 4h). The lowered effluent iron concentrations (0.7 ± 0.5 vs. 1.9 ± 1.7 mg/L) concurrent with the lowered sulfide concentrations suggested more efficient formation of iron sulfide precipitates as a result of the sludge recycling.

Figure 5 presents the means and standard deviations of iron and sulfur mass loads. In the influent ($n = 32$), both sulfur and iron were in dissolved forms with daily loads of 231 mg/day and 404 mg/day, respectively. During baseline operation ($n = 25$), average dissolved sulfur (sulfate and sulfide) in the effluents from the two bioreactors accounted for 7.3% of the influent sulfur load. Effluent particulate sulfur (estimated as sulfate after the sludge samples were acidified and oxidized) accounted for 50.5% of the influent sulfur load to the bioreactors. Similarly, average dissolved iron (total iron in the effluent) in the effluents accounted for only a small fraction of the influent iron load (5.7%) while particulate iron (total iron in the sludge

materials) accounted for a much greater fraction (53%). During the sludge recycling operation ($n = 7$), reduced amounts of both dissolved sulfur and iron (6.4% and 1.5% of the influent load, respectively) were observed compared to the baseline operation. Conversely, both the effluent particulate sulfur and iron showed significant increases (62% and 68%, respectively). In the oxidizing basin ($n = 7$), dissolved sulfur (estimated by sulfate in the filtrate) accounted for a significantly higher percentage of the influent sulfur load (42%) compared to the bioreactor effluents. There was still a substantial but much lower particulate sulfur fraction (30%) compared to the bioreactors. Iron exhibited similar trends in both dissolved and particulate forms (45% and 18%, respectively). These results showed that the oxidation treatment transferred the majority of particulate S and Fe in the sludge materials to oxidized dissolved forms in the oxidizing basin. The unaccounted fractions of the chemical masses (i.e., loss) are attributable to several possible mechanisms including ferrous sulfide precipitation retained in void spaces or associated with the biomass in the bioreactors, evaporative loss of sulfide, and inaccuracy in chemical analyses.

Further analyses of the sludge extracted from the bioreactors (anaerobic sludge) and the oxidized sludge showed similar quantities of total iron. However, the distribution of chemical forms was different. In the anaerobic sludge, the iron was particulate with small percentages of dissolved Fe^{2+} and Fe^{3+} . In the oxidizing basin iron precipitates were partially converted to dissolved forms including Fe^{2+} and Fe^{3+} (Figure 6). Similarly, the sulfide precipitates were converted to dissolved sulfate, and partially oxidized forms including sulfite and thiosulfate.

3.3 Sludge solids evolution over long-term operation

During the 450-day study period (phase I), both VSS and TSS of the sludge materials from the bioreactors varied slightly with the different COD/sulfate and Fe/S ratios (Figure 7a). VSS and TSS concentrations were 761 ± 39 mg/L, and $2,477\pm194$ mg/L, respectively, resulting in a VSS/TSS ratio of 0.31 ± 0.02 . This suggested fairly uniform solid content and composition of the sludge materials even with variations in chemical loading during long-term operation. In phase II, sludge recycling occurrences were found to cause elevations in both VSS and TSS (Figure 7b). The sludge recycling also caused increases in VSS/TSS ratio, indicating relatively higher quantities of VSS than NVSS in the oxidized sludge.

4. Conclusions

This study represents an innovative treatment method with its novelty residing in the use of iron for sulfur recycling.

- The mass balance analysis and chemical analyses of Fe and S at different stages of the treatment process demonstrated that the designed biochemical reactions were successfully carried out and produced satisfactory results.
- With a COD/sulfate mass ration of 2, and a Fe/S molar ratio of 1, this process operated under a condition conducive to sulfidogenic treatment of the wastewater and yielded the best treatment performance among the different chemical loads.
- Sludge oxidation and recycling significantly enhanced treatment performance. The oxidation in the oxidizing basin was found to only partially convert inorganic precipitates to soluble iron and sulfur and can be improved.

- The process exhibited treatment stability with reasonable variations under a range of COD/sulfate and Fe/S ratios. The sludge content was found to be fairly consistent over the long periods of operation without sludge recycling, and the bioreactors were found to efficiently absorb the changes in these material loads caused by the sludge recycling.

Additional studies are required to further optimize the treatment process and elucidate the treatment reactions. In particular, guidelines on C: Fe: S load ratios can be developed for optimized treatment performance. Studies investigating specific biochemical reactions in the sulfidogenic bioreactors and oxidizing basin, and their microbial communities are expected to generate useful results to further develop this treatment method. Further characterization of the sludge materials would also provide insights into the fate of two key chemical elements.

Acknowledgement

This study was partly supported by the National Science Foundation (award number: #1458952). The authors are thankful for the support.

References

- Al Zuhair, S., El-Naas, M.H. and Al Hassani, H.,2008 Sulfate inhibition effect on sulfate reducing bacteria. *Journal of Biochemical Technology* 1(2), 39-44.
- APHA 2005 Standard methods for the examination of water and wastewater. Standard methods for the examination of water and wastewater. 21th ed. Washington: APHA.
- ASTM D1252-06,2006 "Standard Test Methods for Chemical Oxygen Demand (Dichromate Oxygen Demand) of Water.
- Barnes, L., Janssen, F., Sherren, J., Versteegh, J., Koch, R. and Scheeren, P.,1991 A new process for the microbial removal of sulphate and heavy metals from contaminated waters extracted by a geohydrological control system. *Chemical engineering research & design* 69(3), 184-186.
- Bhattacharyya, D., Jumawan, A., Sun, G., Sund-Hagelberg, C. and Schwitzgebel, K.,1981 Precipitation of heavy metals with sodium sulfide: bench-scale and full-scale experimental results.
- Choi, E. and Rim, J.M.,1991 Competition and inhibition of sulfate reducers and methane producers in anaerobic treatment. *Water Science & Technology* 23(7-9), 1259-1264.
- Damianovic, M.H.R.Z. and Foresti, E.,2007 Anaerobic degradation of synthetic wastewaters at different levels of sulfate and COD/sulfate ratios in horizontal-flow anaerobic reactors (HAIB). *Environmental engineering science* 24(3), 383-393.
- Deng, D. and Lin, L.-S.,2013 Two-stage combined treatment of acid mine drainage and municipal wastewater. *Water Science & Technology* 67(5), 1000-1007.
- Deng, D., Weidhaas, J.L. and Lin, L.-S.,2016 Kinetics and Microbial Ecology of Batch Sulfidogenic Bioreactors for Co-treatment of Municipal Wastewater and Acid Mine Drainage. *Journal of hazardous materials*.
- Diekert, G., Balows, A., Truper, H., Dworkin, M., Harder, W. and Schleifer, K.,1992 The acetogenic bacteria. *The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, application*, vol. I. (Ed. 2), 517-533.
- El Bayoumy, M.A., Bewtra, J.K., Ali, H.I. and Biswas, N.,1999 Sulfide production by sulfate reducing bacteria with lactate as feed in an upflow anaerobic fixed film reactor. *Water, Air, and Soil Pollution* 112(1-2), 67-84.
- Emerson, S., Jacobs, L. and Tebo, B.,1983 Trace metals in sea water, pp. 579-608, Springer.
- Friedl, G.F., Mockaitis, G., Rodrigues, J.A., Ratusznei, S.M., Zaiat, M. and Foresti, E.,2009 AnSBBR applied to organic matter and sulfate removal: interaction effect between feed strategy and COD/sulfate ratio. *Applied biochemistry and biotechnology* 159(1), 95-109.
- Hirasawa, J.S., Sarti, A., Del Aguila, N.K.S. and Varesche, M.B.A.,2008 Application of molecular techniques to evaluate the methanogenic archaea and anaerobic bacteria in the presence of oxygen with different COD: Sulfate ratios in a UASB reactor. *Anaerobe* 14(4), 209-218.
- Hoehler, T.M., Alperin, M.J., Albert, D.B. and Martens, C.S.,2001 Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment. *FEMS Microbiology Ecology* 38(1), 33-41.
- Jeong, T.-Y., Cha, G.-C., Seo, Y.-C., Jeon, C. and Choi, S.S.,2008 Effect of COD/sulfate ratios on batch anaerobic digestion using waste activated sludge. *Journal of Industrial and Engineering Chemistry* 14(5), 693-697.
- Johnson, K.L. and Younger, P.L.,2006 The co-treatment of sewage and mine waters in aerobic wetlands. *Engineering Geology* 85(1), 53-61.

Jong, T. and Parry, D.L.,2003 Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. *Water Research* 37(14), 3379-3389.

Karamalidis, A.K., Psycharis, V., Nicolis, I., Pavlidou, E., Benazeth, S. and Voudrias, E.A.,2008 Characterization of stabilized/solidified refinery oily sludge and incinerated refinery sludge with cement using XRD, SEM and EXAFS. *Journal of Environmental Science and Health, Part A* 43(10), 1144-1156.

Lens, P., Visser, A., Janssen, A., Pol, L.H. and Lettinga, G.,1998 Biotechnological treatment of sulfate-rich wastewaters. *Critical Reviews in Environmental Science and Technology* 28(1), 41-88.

McCullough, C., Lund, M. and May, J.,2008 Field-scale demonstration of the potential for sewage to remediate acidic mine waters. *Mine Water and the Environment* 27(1), 31-39.

Metcalf, E., Tchobanoglous, G., Stensel, H.D., Tsuchihashi, R. and Burton, F.,2013 *Wastewater Engineering: Treatment and Resource Recovery*. McGraw-Hill, New York, NY, USA.

Parkin, G. and Speece, R.,1983 Attached versus suspended growth anaerobic reactors: response to toxic substances. *Water Science & Technology* 15(8-9), 261-289.

Postgate, J.R.,1979 *The sulphate-reducing bacteria*, CUP Archive.

Reis, M., Almeida, J., Lemos, P. and Carrondo, M.,1992 Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnology and bioengineering* 40(5), 593-600.

Rinzema, A. and Lettinga, G.,1988 The effect of sulphide on the anaerobic degradation of propionate. *Environmental technology* 9(2), 83-88.

Schippers, A. and Jørgensen, B.B.,2002 Biogeochemistry of pyrite and iron sulfide oxidation in marine sediments. *Geochimica et Cosmochimica Acta* 66(1), 85-92.

Strosnider, W. and Nairn, R.,2010 Effective passive treatment of high-strength acid mine drainage and raw municipal wastewater in Potosí, Bolivia using simple mutual incubations and limestone. *Journal of Geochemical Exploration* 105(1), 34-42.

Strosnider, W., Winfrey, B. and Nairn, R.,2011a Alkalinity generation in a novel multi-stage high-strength acid mine drainage and municipal wastewater passive co-treatment system. *Mine Water and the Environment* 30(1), 47-53.

Strosnider, W.H., Nairn, R.W., Peer, R.A. and Winfrey, B.K.,2013 Passive co-treatment of Zn-rich acid mine drainage and raw municipal wastewater. *Journal of Geochemical Exploration* 125, 110-116.

Strosnider, W.H., Winfrey, B.K. and Nairn, R.W.,2011b Novel passive co-treatment of acid mine drainage and municipal wastewater. *Journal of Environmental Quality* 40(1), 206-213.

Vavilin, V., Fernandez, B., Palatsi, J. and Flotats, X.,2008 Hydrolysis kinetics in anaerobic degradation of particulate organic material: an overview. *Waste management* 28(6), 939-951.

Velasco, A., Ramírez, M., Volke-Sepúlveda, T., González-Sánchez, A. and Revah, S.,2008 Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation. *Journal of hazardous materials* 151(2), 407-413.

Vogels, G.D., Keltjens, J.T. and Van Der Drift, C.,1988 Biochemistry of methane production. *Biology of anaerobic microorganisms*, 707-770.

Vossoughi, M., Shakeri, M. and Alemzadeh, I.,2003 Performance of anaerobic baffled reactor treating synthetic wastewater influenced by decreasing COD/SO₄ ratios. *Chemical Engineering and Processing: Process Intensification* 42(10), 811-816.

Widdel, F.,1988 Microbiology and ecology of sulfate-and sulfur-reducing bacteria. *Biology of Anaerobic Microorganisms*, 469-585.

Winfrey, B., Strosnider, W., Nairn, R. and Strevett, K.,2010 Highly effective reduction of fecal indicator bacteria counts in an ecologically engineered municipal wastewater and acid mine drainage passive co-treatment system. *Ecological Engineering* 36(12), 1620-1626.

Al Zuhair, S., El-Naas, M.H. and Al Hassani, H., 2008. Sulfate inhibition effect on sulfate reducing bacteria. *Journal of Biochemical Technology* 1 (2), 39-44.

APHA 2005. Standard methods for the examination of water and wastewater. Standard methods for the examination of water and wastewater. 21th ed. Washington: APHA.

ASTM D1252-06, 2006. "Standard Test Methods for Chemical Oxygen Demand (Dichromate Oxygen Demand) of Water.

Barnes, L., Janssen, F., Sherren, J., Versteegh, J., Koch, R. and Scheeren, P., 1991. A new process for the microbial removal of sulphate and heavy metals from contaminated waters extracted by a geohydrological control system. *Chemical engineering research & design* 69 (3), 184-186.

Bhattacharyya, D., Jumawan, A., Sun, G., Sund-Hagelberg, C. and Schwitzgebel, K., 1981 Precipitation of heavy metals with sodium sulfide: bench-scale and full-scale experimental results.

Choi, E. and Rim, J.M., 1991. Competition and inhibition of sulfate reducers and methane producers in anaerobic treatment. *Water Science & Technology* 23 (7-9), 1259-1264.

Damianovic, M.H.R.Z. and Foresti, E., 2007. Anaerobic degradation of synthetic wastewaters at different levels of sulfate and COD/sulfate ratios in horizontal-flow anaerobic reactors (HAIB). *Environmental engineering science* 24 (3), 383-393.

Deng, D. and Lin, L.-S., 2013. Two-stage combined treatment of acid mine drainage and municipal wastewater. *Water Science & Technology* 67 (5), 1000-1007.

Deng, D., Weidhaas, J.L. and Lin, L.-S., 2016. Kinetics and Microbial Ecology of Batch Sulfidogenic Bioreactors for Co-treatment of Municipal Wastewater and Acid Mine Drainage. *Journal of hazardous materials*.

Diekert, G., Balows, A., Truper, H., Dworkin, M., Harder, W. and Schleifer, K., 1992. The acetogenic bacteria. The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, application, vol. I. (Ed. 2), 517-533.

El Bayoumy, M.A., Bewtra, J.K., Ali, H.I. and Biswas, N., 1999. Sulfide production by sulfate reducing bacteria with lactate as feed in an upflow anaerobic fixed film reactor. *Water, Air, and Soil Pollution* 112 (1-2), 67-84.

Emerson, S., Jacobs, L. and Tebo, B., 1983 Trace metals in sea water, pp. 579-608, Springer.

Friedl, G.F., Mockaitis, G., Rodrigues, J.A., Ratusznei, S.M., Zaiat, M. and Foresti, E., 2009. AnSBBR applied to organic matter and sulfate removal: interaction effect between feed strategy and COD/sulfate ratio. *Applied biochemistry and biotechnology* 159 (1), 95-109.

Hirasawa, J.S., Sarti, A., Del Aguila, N.K.S. and Varesche, M.B.A., 2008. Application of molecular techniques to evaluate the methanogenic archaea and anaerobic bacteria in the presence of oxygen with different COD: Sulfate ratios in a UASB reactor. *Anaerobe* 14 (4), 209-218.

Hoehler, T.M., Alperin, M.J., Albert, D.B. and Martens, C.S., 2001. Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment. *FEMS Microbiology Ecology* 38 (1), 33-41.

Ingvorsen, K., Zehnder, A.J. and Jørgensen, B.B., 1984. Kinetics of sulfate and acetate uptake by *Desulfobacter postgatei*. *Applied and environmental microbiology* 47 (2), 403-408.

Jeong, T.-Y., Cha, G.-C., Seo, Y.-C., Jeon, C. and Choi, S.S., 2008. Effect of COD/sulfate ratios on batch anaerobic digestion using waste activated sludge. *Journal of Industrial and Engineering Chemistry* 14 (5), 693-697.

Jong, T. and Parry, D.L., 2003. Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. *Water Research* 37 (14), 3379-3389.

Kaksonen, A.H., Franzmann, P.D. and Puhakka, J.A., 2003. Performance and ethanol oxidation kinetics of a sulfate-reducing fluidized-bed reactor treating acidic metal-containing wastewater. *Biodegradation* 14 (3), 207-217.

Karamalidis, A.K., Psycharis, V., Nicolis, I., Pavlidou, E., Benazeth, S. and Voudrias, E.A., 2008. Characterization of stabilized/solidified refinery oily sludge and incinerated refinery sludge with cement using XRD, SEM and EXAFS. *Journal of Environmental Science and Health, Part A* 43 (10), 1144-1156.

Kolmert, Å. and Johnson, D.B., 2001. Remediation of acidic waste waters using immobilised, acidophilic sulfate - reducing bacteria. *Journal of Chemical Technology and Biotechnology* 76 (8), 836-843.

Lens, P., Visser, A., Janssen, A., Pol, L.H. and Lettinga, G., 1998. Biotechnological treatment of sulfate-rich wastewaters. *Critical Reviews in Environmental Science and Technology* 28 (1), 41-88.

McCullough, C., Lund, M. and May, J., 2008. Field-scale demonstration of the potential for sewage to remediate acidic mine waters. *Mine Water and the Environment* 27 (1), 31-39.

Metcalf, E., Tchobanoglous, G., Stensel, H.D., Tsuchihashi, R. and Burton, F., 2013. *Wastewater Engineering: Treatment and Resource Recovery*. McGraw-Hill, New York, NY, USA.

Oude Elferink, S., 1998. Sulfate-reducing bacteria in anaerobic bioreactors.

Parkin, G. and Speece, R., 1983. Attached versus suspended growth anaerobic reactors: response to toxic substances. *Water Science & Technology* 15 (8-9), 261-289.

Postgate, J.R., 1979 *The sulphate-reducing bacteria*, CUP Archive.

Reis, M., Almeida, J., Lemos, P. and Carrondo, M., 1992. Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnology and bioengineering* 40 (5), 593-600.

Rinzema, A. and Lettinga, G., 1988. The effect of sulphide on the anaerobic degradation of propionate. *Environmental technology* 9 (2), 83-88.

Sahinkaya, E., Özkaya, B., Kaksonen, A.H. and Puhakka, J.A., 2007. Sulfidogenic fluidized - bed treatment of metal - containing wastewater at low and high temperatures. *Biotechnology and bioengineering* 96 (6), 1064-1072.

Schippers, A. and Jørgensen, B.B., 2002. Biogeochemistry of pyrite and iron sulfide oxidation in marine sediments. *Geochimica et Cosmochimica Acta* 66 (1), 85-92.

Schönheit, P., Kristjansson, J.K. and Thauer, R.K., 1982. Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. *Archives of microbiology* 132 (3), 285-288.

Strosnider, W., Winfrey, B. and Nairn, R., 2011a. Alkalinity generation in a novel multi-stage high-strength acid mine drainage and municipal wastewater passive co-treatment system. *Mine Water and the Environment* 30 (1), 47-53.

Strosnider, W.H., Nairn, R.W., Peer, R.A. and Winfrey, B.K., 2013. Passive co-treatment of Zn-rich acid mine drainage and raw municipal wastewater. *Journal of Geochemical Exploration* 125, 110-116.

Strosnider, W.H., Winfrey, B.K. and Nairn, R.W., 2011b. Novel passive co-treatment of acid mine drainage and municipal wastewater. *Journal of Environmental Quality* 40 (1), 206-213.

URS Report, 2003 *Passive and semi-active treatment of acid rock drainage from metal mines-state of the practice*.

Velasco, A., Ramírez, M., Volke-Sepúlveda, T., González-Sánchez, A. and Revah, S., 2008. Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation. *Journal of hazardous materials* 151 (2), 407-413.

Vogels, G.D., Keltjens, J.T. and Van Der Drift, C., 1988. Biochemistry of methane production. *Biology of anaerobic microorganisms*, 707-770.

- Vossoughi, M., Shakeri, M. and Alemzadeh, I., 2003. Performance of anaerobic baffled reactor treating synthetic wastewater influenced by decreasing COD/SO₄ ratios. *Chemical Engineering and Processing: Process Intensification* 42 (10), 811-816.
- Widdel, F., 1988. Microbiology and ecology of sulfate-and sulfur-reducing bacteria. *Biology of Anaerobic Microorganisms.*, 469-585.
- Winfrey, B., Strosnider, W., Nairn, R. and Strevett, K., 2010. Highly effective reduction of fecal indicator bacteria counts in an ecologically engineered municipal wastewater and acid mine drainage passive co-treatment system. *Ecological Engineering* 36 (12), 1620-1626.
- Yoda, M., Kitagawa, M. and Miyaji, Y., 1987. Long term competition between sulfate-reducing and methane-producing bacteria for acetate in anaerobic biofilm. *Water Research* 21 (12), 1547-1556.

Fig.1. Fe(II)-dosed sulfidogenic treatment process with sludge oxidation and recycling.

Fig.2. Phase I a) COD removal, b) sulfate reduction, c) iron retention and d) pH and as a function of COD/sulfate mass ratio (1, 1.33, 2, 4, 8) and Fe/S molar ratio (1, 2, 4). Results were obtained with internal recirculation ratio=5.3 and without sludge recycle.

Fig.3. Phase I (a) COD oxidation and (b) sulfate reduction rates estimated by steady-state concentrations of COD and sulfate under a range of COD/sulfate mass ratios (1, 1.33, 2, 4, 8) and Fe/S molar ratios (1, 2, 4) with hydraulic retention time 0.43 days.

Fig.4. Average concentrations of a) COD, b) sulfate as S, c) pH, d) TSS, e) TDS, f) VSS, g) iron, and h) sulfide-S in the two bioreactor effluents during the 62-day operation (phase II). The shaded areas mark the sludge recycling occurrences. The box plots show the statistics of influent (Inf) and effluent quality during the baseline operation (BR) and time periods with sludge recycling (BR/R).

Fig.5. Phase II iron and sulfur mass balance at the different stages of the treatment process, including the influent (Inf), bioreactors without sludge recycle (BR), bioreactors with sludge recycle (BR w/R), and the oxidizing basin (OxB). Legend: S(dis): soluble sulfur, S(par): particulate sulfur, S(loss): total sulfur in influent-S(dis)-S(par), and the same definitions apply for Fe(dis), Fe(par) and Fe(loss).

Fig.6. (a) Phase II iron and (b) sulfur content of the anaerobic and oxidized sludge in particulate (par) and dissolved (dis) forms.

Fig.7. Solids content and VSS/TSS ratio of the (a) sludge materials from the bioreactors in phase I (i.e., without sludge recycling), and (b) sludge materials from the bioreactors on days without sludge recycling and those days with recycling (indicated by the arrows) in phase II.

Table 1 Michaelis-Menten kinetic parameters for COD oxidation by sulfate reducing cultures

Culture	V_{max} mg/(L·d)	K_m mg/L	Reference
Enriched culture from anaerobic digester sludge	475.2	4.3	Deng et al. (2016)
Enriched culture from mining areas	316.8	--	Sahinkaya et al. (2007)
Mixed culture of SRB and methanogens	47.5-50.4	2.7-3.5	Kaksonen et al. (2003)
Mixed culture of SRB and methanogens	936	9.5	Yoda et al. (1987)
<i>Desulfobacter postgatei</i>	576-1728	13.6	Schönheit et al. (1982)
<i>Desulfobacter postgatei</i>	4320-4464	3.8-4.5	Ingvorsen et al. (1984)
<i>Desulfobacter postgatei</i>	10224-13536	NR	Widdel (1988)
<i>Desulforhabdus amnigenus</i>	2448	35	Oude Elferink (1998)
<i>Desulfobacca acetoxidans</i>	3600	35	Oude Elferink (1998)