Revista Mexicana de Ingeniería Química

Sulfate-rich wastewater treatment using an integrated anaerobic/aerobic biological system

Tratamiento de aguas residuales con alto contenido de sulfatos empleando un sistema biológico integrado anaerobio/aerobio

C.D. Loreto-Muñoz[1], F.J. Almendariz-Tapia[1], A.R. Martin-Garcia[1], R. Sierra-Alvarez[2], V. Ochoa-Herrera[3], O. Monge-Amaya [1]*

[1]*Departamento de Ingeniería Química y Metalurgia, Universidad de Sonora. Rosales y Blvd. Luis Encinas. Hermosillo, Sonora, México, C.P. 83000.*

[2]*Department of Chemical and Environmental Engineering, University of Arizona. P.O.Box 210011. Tucson, Arizona, United States, C.P. 85721-0011.*

[3]*Colegio de Ciencias e Ingenierías- El Politécnico, Instituto Biosfera, Universidad San Francisco de Quito. Diego de Robles y Vía Interoceánica, Quito, Ecuador, C.P. 17-1200-841.*

Abstract

This study evaluated the performance of an integrated anaerobic/aerobic (IAAB) system used for the biological treatment of sulfate-rich wastewater through the reduction of sulfate and the oxidation of sulfide to assess its potential for possible acid mine drainage (AMD) treatment. Separately, an anaerobic sludge was adapted to sulfate-reducing conditions, and an aerobic sludge was activated in a glass column with sucrose as carbon source. Once the sludge activation concluded, the IAAB system, composed of an anaerobic sulfate-reducing reactor (ASRr) and an aerobic sulfide-oxidizing reactor (ASOr), was integrated and operated with a sulfate-rich synthetic solution. The reactor operated with increasing organic (2-4 g COD/L•d) and sulfate (0.4-5.97 g SO_4^{2-}/L•d) loading rates and decreasing COD/SO_4^{2-} ratios (5-0.67). During the 43 days of adaptation of the ASRr, a COD/SO_4^{2-} ratio of 1 g/g caused almost a complete displacement of methanogenic activity by sulfate-reducing activity. The removal efficiencies in the IAAB system for COD, sulfate, and produced sulfide were 90%, 60%, and 99%, respectively. By the end of the IAAB system operation, electron flow was mainly directed to sulfidogenic activity, representing 91%. This study demonstrates that a two-step anaerobic/aerobic biological system operating at a high organic loading rate (OLR) can be used for sulfate and organic matter removal from sulfate-rich wastewaters.

Keywords: sulfate-reduction, sulfide-oxidation, percentage of electron equivalents, integrated bioreactors, microbial competition.

Resumen

En este estudio se evaluó el comportamiento de un sistema biológico integrado anaerobio/aerobio (IAAB) para el tratamiento de aguas residuales con alto contenido de sulfatos, para el potencial tratamiento de drenajes ácidos de mina (DAM). Inicialmente un inóculo anaerobio fue adaptado a condiciones sulfato-reductoras y uno aerobio fue activado en una columna de vidrio con sacarosa. Una vez que la activación de los inóculos concluyó, el sistema IAAB, compuesto de un reactor anaerobio sulfato-reductor (ASRr) y un reactor aerobio sulfoxidante (ASOr), fue integrado. El sistema IAAB operó con una solución sintética, con variaciones en la carga orgánica (2-4 g COD/L•d) y sulfatos (0.4-5.97 g SO_4^{2-}/L•d) volumétrica y la relación DQO/SO_4^{2-} (5-0.67). Durante los 43 días de adaptación del ASRr, la relación de 1 g DQO/g SO_4^{2-} permitió un desplazamiento casi completo de la actividad metanogénica. Las eficiencias de remoción de DQO, sulfato, y sulfuro producido en el sistema IAAB fueron 90%, 60% y 99%, respectivamente. Hacia el final de la operación del sistema IAAB, el flujo de electrones fue principalmente hacia actividad sulfidogénica, representando el 91% de la actividad metabólica. Este estudio demostró que un sistema biológico de dos etapas operando a altas cargas orgánicas puede ser utilizado para la remoción de sulfato y materia orgánica.

Palabras clave: sulfato-reducción, sulfoxidación, porcentaje de electrones equivalentes, biorreactores integrados, competencia microbiana.

1   Introduction

Several industries are responsible for the anthropogenic generation of sulfate waste and wastewater. These include food production, molasses, tannery, pulp and paper, dye and detergent manufacturing, cellulosic ethanol production, and mining and metallurgical processes (Blázquez *et al.*, 2016; Shan *et al.*, 2017; Serrano *et al.*, 2020). Mining and metallurgical processes can generate acid mine drainage (AMD), which can cause environmental problems due to its high content of heavy metals and sulfates. AMD is an acid solution generated when sulfur minerals, present in both active and abandoned mine wastes, oxidize with oxygen and water (Delkash *et al.*, 2015; Alcázar-Medina *et al.*, 2020). The result is a leached agent that contains high quantities of sulfuric acid and metals, such as Mn, Cr, Cd, Cu, Zn, Pb, As, with the potential to contaminate surrounding environments (Gao *et al.*, 2019).

Among various AMD treatment alternatives exists the use of sulfate-reducing bacteria (SRB), which obtain energy through dissimilatory reduction of sulfate to sulfide that reacts with metals forming precipitates (Ballester *et al.*, 2017). The main parameters that affect sulfate-reduction are hydraulic retention time (HRT), temperature, pH, oxygen, COD/SO_4^{2-} ratio, sludge type, and organic matter present (Zhao *et al.*, 2017). SRB can use low molecular weight compounds, like acetate, lactate, ethanol, and H_2, as electron donors (Celis-García *et al.*, 2008).

Sulfate-reduction produces H_2S, a corrosive and toxic compound. Sulfide-oxidizing bacteria (SOB) use H_2S to produce elemental sulfur, thiosulfates, sulfites, and sulfates (Meyer *et al.*, 2016). Microorganisms can produce sulfur granules that are nontoxic, noncorrosive, with high sulfur content, that can be employed in bioleaching processes and agricultural production (Cai *et al.*, 2017). Treatment using bioreactors is a great way to remove sulfates and sulfides through biological methods. Sulfidogenic reactors allow AMD treatment by reducing sulfate concentration and metal recovery, even at low pH (2.2-6.5) with various electron donors (Sahinkaya & Yucesoy, 2010, Costa *et al.*, 2017). In contrast, selective sulfide-oxidation for S_0 production is attained by maintaining low or limited oxygen concentrations in reactors (0.2-1.0 mg/L) (Lohwacharin & Annachhatre, 2010; Sun *et al.*, 2017).

Usually, a biological wastewater treatment consists of two separate reactors; however, if dissolved oxygen (DO) levels are manipulated, both processes can occur in a single reactor, as shown by Xu *et al.* (2012). Their results indicate that SOB activity increases, while SRB activity shows no inhibition at 0.10-0.12 mg/L of DO. Sulfur compounds, like SO_4^{2-} and H_2S can be eliminated by integrating the production of sulfides by SRB and the biological or physicochemical sulfide-oxidation to elemental sulfur (Celis-García *et al.*, 2008).

Anaerobic reactors can treat wastewater streams with high organic loading; consequently, their effluent contains low-weight organic matter that can be used by aerobic treatments (Zhang *et al.*, 2014). Integrated systems, with or without physical separation of the anaerobic-aerobic zones, proved higher chemical oxygen demand (COD) removal efficiencies than single-step configurations (Chan *et al.*, 2009). Therefore, the integration of both processes can be a feasible alternative over conventional technologies, like inverse osmosis and neutralization, for wastewater treatment, given they improve the removal of pollutants by combining anaerobic, aerobic, and anoxic processes (Plascencia-Jatomea *et al.*, 2015). Some examples of contaminated effluents treated in integrated or hybrid reactors are emulsion wastewaters (Zhang *et al.*, 2014), textile effluents containing recalcitrant azo-dyes (Gadow & Li, 2020), and acrylic acid wastewaters (Show et al., 2020).

Sulfate and sulfide removal in waste streams have been widely studied, but almost no studies have focused on combining both processes in a single reactor. Therefore, this study aims to evaluate the feasibility of using a two-step biological anaerobic/aerobic reactor to treat synthetic sulfate-rich wastewater for its possible future use in AMD treatment, analyzing the effect of the organic loads in sulfate, sulfide, and organic matter removal. The IAAB configuration is expected to produce sulfides in the anaerobic region and elemental sulfur in the aerobic region. If the produced sulfide in the ASRr is sufficient, it can react with metals, forming metallic sulfides. These can precipitate and be removed from contaminated streams, like AMD, for safe disposal in water streams. Sulfides that do not react will enter the ASOr. There it is expected to transform to elemental sulfur. Elemental sulfur is the desired final product in the IAAB system effluent. It is a less toxic sulfur compound than sulfide and sulfate and has potential industrial uses if recovered as a precipitate.

2 Materials and methods

2.1 Sludge

Three samples of anaerobic sludge were characterized based on total suspended solids (TSS), volatile suspended solids (VSS), specific methanogenic activity (SMA), and sulfide generation activity (SGA) (Andrade & Ochoa-Herrera, 2013). Sludge A was activated in an up-flow anaerobic sludge bed (UASB) reactor with a COD/SO_4^{2-} ratio of 5 g/g. Sludge B was a granular sludge obtained from an anaerobic bioreactor treating effluents from a brewery company. Sludge C was previously used in a methanogenic process. All sludges were collected and stored under anaerobic conditions in plastic gallons. The aerobic sludge was collected from a municipal wastewater treatment plant in Hermosillo, Sonora, Mexico. The VSS/TSS ratio of the microbial aerobic sludge was 44.62%.

2.2 Batch bioassays

Batch bioassays were conducted in triplicates using 160 mL glass serum flasks with rubber stoppers and aluminum crimp seals. Separate assays with samples of each anaerobic sludge containing 1.5 g VSS/L and 100 mL of mineral medium, 50 mL headspace, with 2 g COD/L served as batch digesters for SMA. Like SMA, individual studies with each sludge were employed to determine SGA, 10 g (9.28 g VSS/L) of the anaerobic sludge, and 100 mL of the mineral medium ratio of 1 $gCOD/gSO_4^{2-}$ were used. Flasks lacking microorganisms were also incubated and served as abiotic controls for bioassays. The headspace was flushed with N_2 gas to assure anaerobic conditions and incubated at 30±2 ℃. Methane, sulfate, and sulfide analysis were performed daily. Sodium acetate (2000 mg/L) was added as the carbon source for both SMA and SGA bioassays. Sulfate was only added to SGA bioassays as anhydrous sodium sulfate (2000 mg SO_4^{2-}/L). The culture medium used for SMA and SGA batch bioassays contained (mg/L): NH_4Cl (280), KH_2PO_4 (195), $MgSO_4 \bullet 7H_2O$ (49), $CaCl_2 \bullet 2H_2O$ (50), $NaHCO_3$ (3000), yeast extract (10), and 1 ml/L of trace element solution. The trace element solution contained (mg/L): H_3CO_3 (50), $FeCl_2\bullet4H_2O$ (2000), $ZnCl_2$ (50), $MnCl_2 \bullet 4H_2O$ (50), $(NH)_6\bullet6H_2O$ (50), $CuCl_2\bullet2H_2O$ (30), $NaSeO_3\bullet5H_2O$ (100), EDTA (1000), resazurin (200), and 36% HCl (1 mL/L) (Ochoa-Herrera *et al.*, 2011).

2.3 Adaptation of the anaerobic sulfate-reducing bioreactor (ASRr)

Sludge B presented the best results in terms of sulfate-reducing activity during batch bioassays; therefore, it was selected to conduct continuous studies. Biomass was adapted in an up-flow anaerobic fixed bed reactor to sulfate-reducing conditions, variating COD/SO_4^{2-}, as shown in Table 1. Deactivated zeolite, with a particle size of 2 mm, pore diameter 3.22×10^{-3} μm, and a Si/Al ratio of 4.53, was employed as biomass support, occupying 60% of the volume of the reactor. The ASRr consisted of an acrylic column with a 6.85-cm internal diameter and 45-cm height. The reactor had an operating volume of 1.5 L and was inoculated with 33% of the working volume with liquified Sludge B. Daily pH, temperature, COD, SO_4^{2-}, and CH_4 concentrations were determined.

The culture medium used in the ASRr and the integrated anaerobic/aerobic biological system (IAAB) contained (mg/L): NH_4Cl (1045), KCl (270), KH_2PO_4 (170), $MgSO_4\bullet7H_2O$ (185), $CaCl_2\bullet2H_2O$ (50), $NaHCO_3$ (2000), yeast extract (20), and 1 mL/L of trace element solution, mentioned previously. COD in the influent was maintained at 2 g COD/L, with glucose as a carbon source.

2.4 Aerobic sludge activation

The aerobic sludge was activated in a fluidized bed reactor packed with synthetic polyamide and polyurethane sponge cut into 1 cm (d.) pieces. Air was supplied using an air pump with plastic diffusers placed in the bottom region of the reactor. The reactor had an internal diameter of 6.85-cm and was 45-cm tall, with an operating volume of 1.5 L. Daily pH, temperature, and COD concentrations were determined. The purpose of the aerobic activation stage was for biomass to completely adhere to the sponge and promote microorganism growth. Once COD removal stabilized, the reactor was coupled to the ASRr using an aeration chamber for adaptation to sulfide-oxidizing conditions. The culture medium used during the activation of the aerobic sludge contained (mg/L): NH_4Cl (320.9), KH_2PO_4 (109.8), $MgSO_4\bullet7H_2O$ (318.6), $CaCl_2\bullet2H_2O$ (66.46), $NaHCO_3$ (1000), yeast extract (100), sucrose (1500), and 1 mL/L of trace element solution, mentioned previously.

2.5 Integrated anaerobic/aerobic biological system (IAAB)

Once both reactors reached steady operation, the ASOr was integrated into the ASRr, forming the IAAB (Fig. 1). Both systems were coupled using an air chamber (10-cm high) between both reactors to separate the anaerobic and aerobic regions. This chamber contained four plastic diffusers connected to an air pump. To ensure a uniform dissolved oxygen concentration in the ASOr an aeration reflux was added using a glass chamber with a fritted glass diffuser connected to a second air feed. This reflux helped avoid the formation of preferential flux channels in the reactor, ensuring complete oxygenation in the upper region of the reactor. The complete IAAB system was 1-m high with a 6.85-cm internal diameter. The total operating volume from the inlet to the outlet of the IAAB system was 3 L.

The IAAB system worked with ascending flow. The raw influent entered the lower region of the system in the ASRr inlet. The synthetic sulfate-rich solution was expected to transform into sulfide compounds (H_2S, HS^-, and S^{2-}) in the ASRr. The effluent of the ASRr entered the aeration chamber, where it came in contact with oxygen. Afterward, it entered the ASOr, where sulfide compounds were expected to sulfide-oxidize to elemental sulfur (S^0). Finally, the treated effluent exited the reactor from the ASOr outlet placed in the upper region of the system. As mentioned previously, two refluxes were added to the IAAB, one in each reactor.
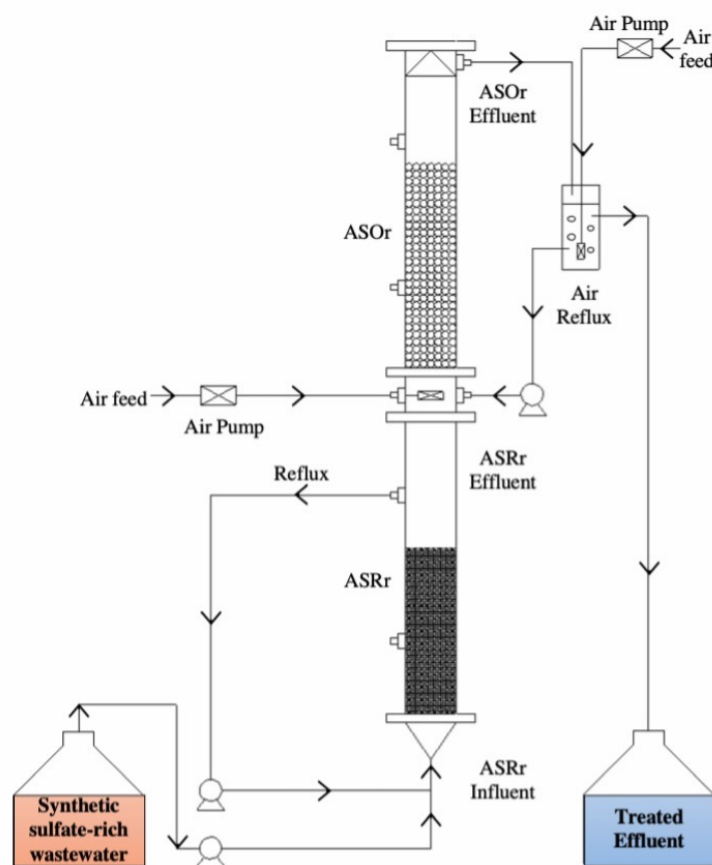

Fig. 1. Schematic representation of the integrated anaerobic/aerobic biological system (IAAB).

Table 1. Conditions maintained and the average concentration of organic components in the influent during the ASRr and IAAB system operation.

Parameter	ASRr			IAAB		
	Period I	Period II	Period III	Period IV	Period V	Period VI
Time (d)	0 - 9	10 - 27	28 - 43	44 - 54	55 - 63	64 - 133
pH	7 - 8	7 - 8	7 - 8	7 - 8	7 - 8	7 - 8
OLR (gCOD/L·d)	2	2	2	1	2	4
SLR (gSO$_4^{2-}$/L·d)	0.4	0.8	2	1	2.99	5.97
Temperature (°C)	37	37	37	37	37	37
COD/SO$_4^{2-}$ Ratio	5	2.5	1	1	0.67	0.67
HRT$_{ASRr}$ (h)	24	24	24	24	24	12
HRT$_{IAAB}$ (h)	-	-	-	48	48	24

The operational conditions of the IAAB are described in Table 1, organic loading rates (ORL) and COD/SO$_4^{2-}$ ratios were varied in this study. Daily COD, SO$_4^{2-}$, and S^{2-} concentrations, temperature, and pH were analyzed. Samples were collected from the lower, intermediate, and upper regions of the reactor. Electron equivalents (%H$_2$S-COD) were determined using equations 1 and 2.

2.6 Calculations

The percentage of electron equivalents was determined using equations 1 and 2 (Sierra-Alvarez *et al.*, 2007):

$$\%\text{CH}_4 - COD = 100 \times \frac{M \bullet F_m}{COD_R} \quad (1)$$

$$\%\text{H}_2\text{S} - COD = 100 \times \frac{S \bullet F_s}{COD_R} \quad (2)$$

where: M= methanogenesis (g CH$_4$/L•d); S= sulfide generation (g S^{2-}/L•d); COD_R= organic matter removal rate (g COD/L•d); and stoichiometric ratios F_m= 4 g CH$_4$-COD/g CH$_4$; and F_s= 2 g S^{2-}-COD/g S.

The COD_R was calculated as follows:

$$COD_R = \frac{COD_{in} - COD_f}{t} \quad (3)$$

COD_{in} and COD_f are the initial and final concentrations of COD concentrations in the batch bioassays, and t is the number of days. When the COD_R is determined in continuous studies, it becomes the slope in COD removal in the ASRr, for each period.

The observed organic matter (%COD_{ASRr}, Eq. 4) and sulfate (%$SO_4^{2-}{}_{IAAB}$, Eq. 5) removal efficiencies in the ASRr were calculated as follows:

$$\%COD_{ASEr} = 100 \times \left(\frac{I_{COD,ASRr} - E_{COD,ASRr}}{I_{COD,ASRr}} \right) \quad (4)$$

$$\%SO_4^{2-}{}_{IAAB} = 100 \times \left(\frac{I_{SO_4^{2-},ASRr} - E_{SO_4^{2-},ASRr}}{I_{SO_4^{2-},ASRr}} \right) \quad (5)$$

where $I_{COD,ASRr}$, and $E_{COD,ASRr}$ are the organic matter (COD) concentrations at the inlet and outlet of the $ASRr$, $I_{SO_4^{2-},ASRr}$ and $E_{SO_4^{2-},ASRr}$ are the sulfate concentrations at the inlet and outlet of the $ASRr$. Similarly, the organic matter removal efficiency in the ASOr (%COD_{ASOr}) was determined using the COD concentrations of the inlet and outlet of the ASOr.

The overall organic matter (%COD_{IAAB}, Eq. 6) and sulfate (%$SO_4^{2-}{}_{IAAB}$, Eq. 7) removal efficiencies of the IAAB system were calculated using the following equations:

$$\%COD_{IAAB} = 100 \times \left(\frac{I_{COD,ASRr} - E_{COD,ASOr}}{I_{COD,ASRr}} \right) \quad (6)$$

$$\%SO_4^{2-}{}_{IAAB} = 100 \times \left(\frac{I_{SO_4^{2-}{}_{IAAB}} - E_{SO_4^{2-}{}_{ASOr}}}{I_{SO_4^{2-}{}_{ASRr}}} \right) \quad (7)$$

where $I_{COD,ASRr}$ is the organic matter concentration in the inlet of the ASRr, $E_{COD,ASOr}$ is the organic matter concentration in the effluent of the ASOr, $I_{SO_4^{2-}{}_{IAAB}}$ is the sulfate concentration in the inlet of the ASRr, and $E_{SO_4^{2-}{}_{IAAB}}$ is the sulfate concentration in the effluent of the ASOr.

Sulfide removal efficiency (%S^{2-}_{ASOr}) was only determined in the ASOr, and was calculated as follows:

$$\%S^{2-}_{ASOr} = 100 \times \left(\frac{I_{S^{2-},ASOr} - E_{S^{2-},ASOr}}{I_{S^{2-},ASOr}} \right) \quad (8)$$

where $I_{S^{2-},ASOr}$ is the sulfide concentration in the inlet of the ASOr, or the sulfide concentration in the effluent of the ASRr. $E_{S^{2-},ASOr}$ is the sulfide concentration in the effluent of the ASOr, or the sulfide concentration in the effluent of the IAAB.

2.7   Analytical methods

A Thermo Scientific Orion 2665 potentiometer was employed for pH measurement. Methane production was measured every 24 hours by displacement of a 3% NaOH solution. COD was determined using a HACH kit, with samples centrifuged for 2500 rpm for sulfide elimination. Sulfate and total sulfide (H_2S, HS^- and S^{2-}) concentrations were determined according to the turbidimetric method and methylene blue method both from the Standard Methods for the Examination of Water and Wastewater (2012).

3   Results and discussion

3.1   Batch bioassays

The VSS/TSS ratios were 69.4% (Sludge A), 56.5% (Sludge B), and 65.1% (Sludge C). All sludges presented low VSS/TSS ratios; a ratio between 0.60 and 0.80 is recommended for anaerobic processes (Metcalf & Eddy, 2014). Fig. 2 represents the time course cumulative methane and sulfide production for all three sludges and abiotic controls using acetate. There was no methane formation in the abiotic control, but production in the treatment bioassays increased with time. Sludge A showed the highest SMA (0.094 g CH_4-COD/g VSS•d), followed by Sludge C (0.056 g CH_4-COD/g VSS•d), while Sludge B presented the lowest SMA (0.044 g CH_4-COD/g VSS•d). Higher SMA was expected for both Sludges A and C, considering they come from reactors employed in wastewater treatment. In these systems, methanogenic microorganism growth is stimulated (Andrade & Ochoa-Herrera, 2013). Additionally, Sludge A was activated using a high COD/SO_4^{2-} ratio.
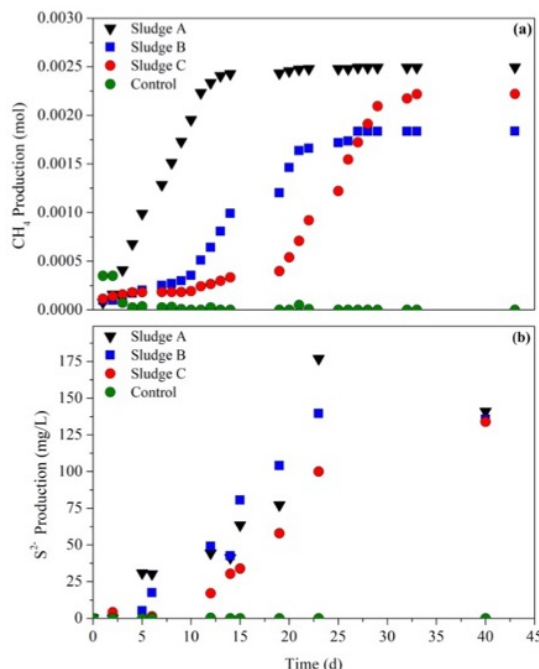

Fig. 2. Time course (a) methane and (b) sulfide production of the anaerobic sludges in the presence of sodium acetate in batch bioassays.

Studies demonstrate that higher ratios promote methanogenic over sulfidogenic activity. Theoretically, a COD/SO_4^{2-} ratio of 0.67 g/g stoichiometrically sufficient for complete organic matter oxidation; ratios of 1 to 2 g/g allow the predominance of sulfidogenesis, while methanogenesis predominates at ratios above this level (Camiloti *et al.*, 2014). However, the overall SMA in all three sludges is below the average reported for acetoclastic methanogenic activity, which is 0.45 gCH_4-COD/g VSS•d (Gallegos-Garcia *et al.*, 2010).

Similar to the methanogenic assays, no sulfide production was observed in the abiotic control. SGA was 0.42, 1.02, and 0.59 mg S^{2-}/gVSS•d, for sludges A, B, and C, respectively. Lu *et al.* (2017) obtained a sulfidogenic activity of 0.41 g S^{2-}-COD/g VSS•d, which is significantly higher than the activities obtained in this study under similar conditions. The low sulfidogenic activity might be attributed to the low content of SRB in the sludge (González-Paz *et al.*, 2020). Given that the VSS represents the fraction of TSS present as biomass, all three sludges presented a low VSS/TSS ratio (Garzón *et al.*, 2015). Therefore, there is a low concentration of microorganisms in the sludge, resulting in lower

metabolic activities. Low metabolic activities could cause prolonged periods of adaptation for a complete displacement of methanogenic activity by sulfidogenic activity.

3.2 Adaptation of the ASRr

The anaerobic consortium, Sludge B from batch bioassays, was adapted to sulfate-reducing conditions in the ASRr, for 43 days using a synthetic effluent. Organic matter and sulfate removal efficiencies are shown in Table 2. Organic matter removal (Fig. 3a) in the ASRr decreased, from 81.2% to 65.1%, with the reduction of the COD/SO_4^{2-}, but sulfate removal increased (Fig. 3b), reaching the highest removal in Period III (72.5%).
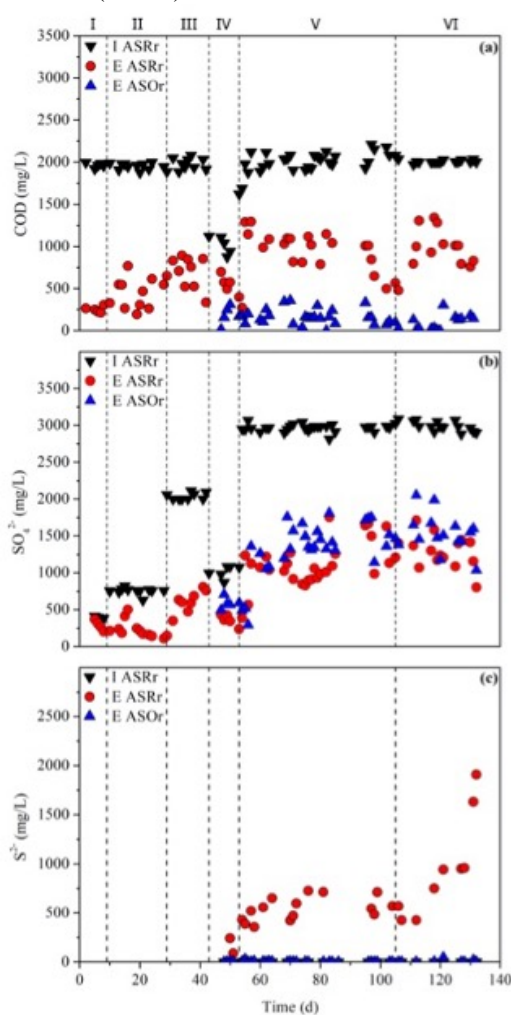

Fig. 3. (a) Organic matter; (b) sulfate; (c) sulfide concentrations in the influent and effluent of the ASRr (Periods I-III) and IAAB (Periods IV-VI) as a function of time.

The increase in sulfate removal is related to the increase in sulfate concentration in the inlet of the reactor, increasing the reaction rate between SRB and sulfate. The increase in the SRB community causes a decrease in substrate microbial competition by the displacement of other microorganisms present in the sludge. At higher COD/SO_4^{2-} ratios, there is increased competition for carbon sources between SRB and methane-producing archaea (MPA); however, as the ratio decreases, SRB growth is favored, explaining the decrease in COD removal and increase in sulfate removal rates (Liu *et al.*, 2018).

Due to inconsistencies in methane measurements, electron equivalents in Period II were not calculated; however, a comparison of the methane production in Periods I (%CH$_4$-COD= 79.6) and III (%CH$_4$-COD=39.2) indicates a clear drop in methanogenic activity. COD biodegradation pathways are related to COD/SO_4^{2-} ratios. For example, at ratios below 1.5 g/g, COD consumption was mainly directed towards sulfate conversion to sulfide, ratios between 1-3 g/g allowed a balance between MPA and SRB, and ratios above 3.5 g/g resulted in the decrease of the sulfate-reducing activity (Jeong *et al.*, 2008; Yuan *et al.*, 2015). The adaptation period of the ASRr shows that electron flow shifted towards methanogenic activity at higher COD/SO_4^{2-} ratios (Period I).

3.3 Integrated anaerobic/aerobic biological system (IAAB)

The aerobic sludge activation period lasted 20 days, reaching a COD removal of 94.1%. This period took place in a separate acrylic reactor previously described. Once COD removal stabilized and the biomass completely adhered to the sponge, the reactor was coupled to the ASRr, forming the IAAB system. The highest COD removal efficiency was observed in Period IV (%COD$_{IAAB}$ = 82.4%), but high removal efficiencies exceeding 80% were maintained during all three periods. Also, COD_R for Periods V (COD_R = 109.2 mg COD/L•d) and VI (COD_R = 109.8 mg/L•d) did not vary significantly with HRT_{IAAB} decrease, indicating that HRT had no considerable effect in COD removal. Accordingly, the aerobic treatment, or ASOr, was conducted to treat the remaining organic matter present in the ASRr, reaching a maximum COD removal of 84.7% in Period VI (Fig. 3a). Therefore, the effluent from the anaerobic treatment has sufficient amounts of soluble organics and solids that can be used by aerobic treatments (Gadow & Li, 2020).

Table 2. Average sulfate and COD removal and percentage of electron equivalents for methane and sulfide production in the ASRr and IAAB.

			Period					
			Individual reactors			Integrated System		
Parameter		Units	I	II	III	IV	V	VI
ASRr	**COD Removal**	mgCOD/L	1712.03	1512.29	1281.63	569.84	1041.21	1116.71
		$\%COD_{ASRr}$	87.19	77.45	65.05	47.95	51.98	55.43
	SO_4^{2-} Removal	mg $\%SO_4^{2-}$/L	106.91	519.13	1477.05	359.29	1089.24	1284.26
		$\%SO_{4\ ASRr}^{2-}$	27.57	69.09	72.5	75.17	58.19	57.25
	H_2S Production	mgS^{2-}/L	-	-	-	196.28	502.42	678.18
		COD_R	102.31	32.18	60.98	50.02	109.26	109.88
	%CODin	$\%CH_4$-COD	79.61	-	39.26	-	-	-
		$\%H_2S$-COD	-	-	-	73.92	85.92	90.92
ASOr	**COD Removal**	mgCOD/L	-	-	-	368.67	761.43	807.24
		$\%COD_{ASRr}$	-	-	-	64.81	76.81	84.69
	H_2S Removal	mgS^{2-}/L	-	-	-	193.54	499.51	669.64
		$\%S_{AS Or}^{2-}$	-	-	-	98.4	100	98.74
IAAB	**COD Removal**	mgCOD/L	-	-	-	938.51	1802.6	1923.95
		$\%COD_{IAAB}$	-	-	-	82.42	90.09	95.61
	$\%SO_4^{2-}$ - Removal	mg $\%SO_4^{2-}$/L	-	-	-	631.06	1417.66	1512.27
		$\%SO_{4\ IAAB}^{2-}$	-	-	-	61.24	48.61	49.08

In Period IV, sulfate removal efficiency reached 75.1% (Table 2) but decreased once the COD/SO_4^{2-} ratio changed to 0.67, probably caused by a substrate or product inhibition, as explained by Xingyu *et al.* (2013). It appeared that the lowering in the COD/SO_4^{2-} ratio could have caused a deterioration in sulfate reduction. H_2S has a critical effect on anaerobic treatments since it can penetrate cells, and in some cases, destroys bacteria, including SRB (Zhou & Xing, 2015). In this study, H_2S (pKa$_1$ = 6.9) and HS$^-$ (pKa$_2$ = 12.9) are expected to be the predominant sulfide species for the conditions employed (Martins *et al.*, 2011). Reported IC50 of undissociated sulfide to SRB at pH levels comparable to those in this study range 69-223 mgH_2S/L (Visser *et al.*, 1996, O'Flaherty *et al.*, 1998). The concentration of undissociated H_2S exceeded these IC50 values during periods of reactor operation. AS shown in Figure 3, the total sulfide levels in ASRr during Period V and VI averaged 502-678 mgS^{2-}/L, respectively. Assuming pH = 7 and pKa$_1$ = 7 (dissociation of H_2S to HS$^-$), the average concentrations of undissociated H_2S in Period V and VI were 251 and 339 mg/L. Although the minimal COD requirement for sulfate reduction is 1 g-COD per 1.5 g-SO_4^{2-} reduced (0.67 COD/SO_4^{2-}), recent studies demonstrate that increasing COD/SO_4^{2-} ratios in the range of 0.67 to 2.5 increases sulfate removal (Yuan

et al., 2015). Sulfate removal (%SO_4^{2-}ASRr = 57-58%) was stable with OLR increase (Period V and VI). In this case, OLR increase with HRT_{IAAB} does not affect the reactor's overall performance, showing no alteration in the reactor's metabolic processes. An increase in HRT can induce a shift in the substrate profile in the outer layer of the granules. This allows the contact of un-adapted bacteria with the contaminant and sometimes can cause inhibition in their metabolic activities. It is imperative to maintain a stable operation in continuous systems to avoid 'shock loading' (Show *et al.*, 2020).

The IAAB system configuration prevented methane quantification, but the percentage of electrons used for sulfidogenesis showed an apparent increase with the low COD/SO_4^{2-} ratios and OLR increase. Low COD/SO_4^{2-} ratios cause predominance in SRB electron usage; however, the proportion of electron flow employed for methanogenic and sulfidogenic routes depends on COD/SO_4^{2-} ratios and operational parameters, like substrates and HRT (Lu *et al.*, 2016). Sulfide-oxidation was determined by sulfide concentrations in the effluent of the ASOr. Although sulfide concentration in the effluent of the ASOr is close to 0 mg/L, sulfate concentrations did not increase significantly compared to the ASRr effluent, indicating an incomplete sulfide-oxidation. Sulfide

loss can be attributed to incomplete oxidation or volatilization. Produced sulfide in the ASRr might have transformed into intermediate compounds in the ASOr like polysulfides, sulfite, and thiosulfates. Oxygen availability could have affected the final sulfur products involved in the ASOr. Sulfur is expected to be the primary end product of biological sulfide-oxidation for oxygen concentrations below 0.1 mg/L, with partial oxidation to thiosulfate. With higher oxygen availability, sulfate is expected to be the predominant end-product (Pokorna-Krayzelova *et al.*, 2018). The amount of dissolved oxygen can be associated with the aeration rate and the use in the biochemical reaction; hence, optimizing aeration is crucial to prevent low or incomplete sulfide-oxidation (Pokorna & Zabranska, 2015). In order to limit sulfide oxidation to elemental sulfur without proceeding entirely to sulfate production, a low oxygen concentration needs to be maintained in the reactor. Additionally, conventional bubbling aeration may strip hydrogen sulfide from the water, affecting sulfur recovery efficiency (Sun *et al.*, 2017). $H_2S(aq)$ acts as a week acid with near-neutral pKa values; in its propionate form, it is volatile and can escape as a gas into the atmosphere in natural or artificial sulfidic systems such as tidelands, paddy fields, and sewage (Sim *et al.*, 2019). Hydrogen sulfide volatilization can also be accounted for sulfide concentration decrease.

Conclusions

The election of a proper microbial consortium is crucial for the development of a wastewater treatment system. Sludge B presented the highest SGA and the lowest SMA from batch studies, indicating a facility to adapt to sulfate-reducing conditions.

COD/SO_4^{2-} ratio has a significant impact on metabolic activity. The 43 days of adaptation of the ASRr, showed that intermediate ratios allow a balance between MPA and SRB. However, lower ratios cause almost a complete displacement of methanogenic activity by sulfidogenic activity.

For the correct integration of two separate reactions into one system, process control and operational conditions are crucial for synchronized operation. The removal efficiencies in the IAAB for COD, sulfate, and sulfide were 90%, 60%, and 99%, respectively. By the end of the operation of the reactor, the percentage of electron equivalents was mainly towards sulfidogenic activity (%H$_2$S-

COD= 90.9). This study demonstrates that a two-step anaerobic/aerobic biological system operating at high OLR can be used for sulfate and organic matter removal from sulfate-rich wastewater. We aim for efficient wastewater treatment with this specific reactor configuration, heavy metal recovery as metal sulfides, and biological elemental sulfur recovery. We understand that further research is still needed to prove its efficiency for AMD treatment, including analyzing pH and heavy metal effect in microorganism growth, H_2S production, and reactor operation. Additional research is needed to extract elemental sulfur and metal precipitates efficiently.

Acknowledgements

Nomenclature

AMD	Acid mine drainage
SRB	Sulfate-reducing bacteria
SOB	Sulfide-oxidizing bacteria
MPA	Methane producing archaea
WWTP	Wastewater treatment plant
UASB	Up-flow anaerobic sludge bed reactor
FBR	Fixed bed reactor
VSS	Volatile suspended solids
TSS	Total suspended solids
ASRr	Anaerobic sulfate-reducing reactor
ASOr	Aerobic sulfide-oxidizing reactor
IAAB	Integrated anaerobic/aerobic biological system
COD	Chemical oxygen demand
HRT	Hydraulic retention time
COD_R	Organic matter removal rate (g COD/L•d)
ORL	Organic loading rate (g COD/L•d)
SLR	Sulfate loading rate (g SO_4^{2-}/L•d)
%CH$_4$-COD	Percentage of electron equivalents employed for methanogenesis
%H$_2$S-COD	Percentage of electron equivalents employed for sulfidogenesis
M	Methanogenesis (g CH$_4$/L•d)
S	Sulfidogenesis (g S^{2-}/L•d)
F_s	F_s= 2 g S^{2-}-COD/g S
F_m	4 g CH$_4$-COD/g CH$_4$

%COD	Organic matter removal efficiency
%SO$_4^{2-}$	Sulfate removal efficiency
%S^{2-}	Sulfide removal efficiency
I	Influent (COD, SO$_4^{2-}$, or S^{2-}) concentration
E	Effluent (COD or SO$_4^{2-}$, or S^{2-}) concentration

Subscripts

in	Initial
f	Final
ASRr	Anaerobic Sulfate-reducing reactor
ASOr	Aerobic Sulfide-oxidizing reactor
IAAB	Integrated Anaerobic/Aerobic biological system
COD	Chemical oxygen demand
SO$_4^{2-}$	Sulfate
S^{2-}	Sulfide

References

Alcázar-Medina, F. A., Núñez-Núñez, C. M., Villanueva-Fierro, I., Antileo, C., & Proal-Nájera, J. B. (2020). Removal of heavy metals present in groundwater from a northern Mexico mining community using *Agave tequilana* Weber extracts. *Revista Mexicana de Ingeniería Química 19*, 1187-1199. http://www.redalyc.org/articulo.oa?id=62029966013

American Public Health Association (Ed.). (2012). *Standard Methods for the Examination of Water and Wastewater (22nd ed.)*.

Andrade, V., & Ochoa-Herrera, V. (2013). Effect of the substrate as electron donor during the microbial sulfate reduction and its possible applications in the biological treatment of acid mine drainage. *Avances En Ciencias e Ingenierías 5*, C31-C38.

Ballester, A., Castro, L., Clara, M., Carlier, J., García-roig, M., Pérez-galende, P., Alvarez, A., Bertagnolli, C., & Guibal, E. (2017). Design of remediation pilot plants for the treatment of industrial metal-bearing effluents (BIOMETAL DEMO project): Lab tests. *Hydrometallurgy 168*, 103-115. https://doi.org/10.1016/j.hydromet.2016.10.010

Blázquez, E., Gabriel, D., Baeza, J. A., & Guisasola, A. (2016). Treatment of high-strength sulfate wastewater using an autotrophic biocathode in view of elemental sulfur recovery. *Water Research 105*, 395-405. https://doi.org/10.1016/j.watres.2016.09.014

Cai, J., Zheng, P., Qaisar, M., & Zhang, J. (2017). Elemental sulfur recovery of biological sulfide removal process from wastewater: A review. *Critical Reviews in Environmental Science and Technology 47*, 2079-2099. https://doi.org/10.1080/10643389.2017.1394154

Camiloti, P. R., Mockaitis, G., Domingues Rodrigues, J. A., Rissato Zamariolli Damianovic, M. H., Foresti, E., & Zaiat, M. (2014). Innovative anaerobic bioreactor with fixed-structured bed (ABFSB) for simultaneous sulfate reduction and organic matter removal. *Journal of Chemical Technology and Biotechnology 89*, 1044-1050. https://doi.org/10.1002/jctb.4199

Cao, J., Zhang, G., Mao, Z. S., Li, Y., Fang, Z., & Yang, C. (2012). Influence of electron donors on the growth and activity of sulfate-reducing bacteria. *International Journal of Mineral Processing 106-109*, 58-64. https://doi.org/10.1016/j.minpro.2012.02.005

Celis-García, L. B., González-Blanco, G., & Meraz, M. (2008). Removal of sulfur inorganic compounds by a biofilm of sulfate reducing and sulfide oxidizing bacteria in a down-flow fluidized bed rector. *Journal of Chemical Technology & Biotechnology May*, 260-268. https://doi.org/10.1002/jctb.1802

Chan, Y. J., Chong, M. F., Law, C. L., & Hassell, D. G. (2009). A review on anaerobic-aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal 155*, 1-18. https://doi.org/10.1016/j.cej.2009.06.041

Costa, J. M., Rodriguez, R. P., & Sancinetti, G. P. (2017). Removal sulfate and metals Fe^{+2}, Cu^{+2}, and Zn^{+2} from acid mine drainage in an anaerobic sequential batch reactor. *Journal of Environmental Chemical Engineering 5*, 1985-1989. https://doi.org/10.1016/j.jece.2017.04.011

Delkash, M., Ebrazi Bakhshayesh, B., & Kazemian, H. (2015). Using zeolitic adsorbents to

cleanup special wastewater streams: A review. *Microporous and Mesoporous Materials 214*, 224-241. https://doi.org/10.1016/j.micromeso.2015.04.039

Gadow, S. I., & Li, Y. Y. (2020). Development of an integrated anaerobic/aerobic bioreactor for biodegradation of recalcitrant azo dye and bioenergy recovery: HRT effects and functional resilience. *Bioresource Technology Reports 9*, 100388. https://doi.org/10.1016/j.biteb.2020.100388

Gallegos-Garcia, M., Celis, L. B., & Razo-Flores, E. (2010). Competencia por sustrato durante el desarrollo de biomasa sulfatorreductora a partir de un lodo metanogénico en un reactor UASB. *26*, 109-117.

Gao, P., Sun, X., Xiao, E., Xu, Z., Li, B., & Sun, W. (2019). Characterization of iron-metabolizing communities in soils contaminated by acid mine drainage from an abandoned coal mine in Southwest China. *Environmental Science and Pollution Research 26*, 9585-9598. https://doi.org/10.1007/s11356-019-04336-6

Garzón, P., Ochoa-Herrera, V., & Peñafiel, R. (2015). Estudio de la generación de gas metano a partir del agua residual del proceso de extracción de aceite de crudo de palma en biodigestores experimentales. *Avances En Ciencias e Ingenierías*, C130-C137.

González-Paz, J. R., Ordaz, A., Jan-Roblero, J., Fernández-Linares, L. C., & Guerrero-Barajas, C. (2020). Sulfate reduction in a sludge gradually acclimated to acetate as the sole electron donor and its potential application as inoculum in a microbial fuel cell. *Revista Mexicana de Ingeniería Química 19*, 1053-1069. http://www.redalyc.org/articulo.oa?id=62029966013

Jeong, T. Y., Cha, G. C., Seo, Y. C., Jeon, C., & Choi, S. S. (2008). Effect of COD/sulfate ratios on batch anaerobic digestion using waste activated sludge. *Journal of Industrial and Engineering Chemistry 14*, 693-697. https://doi.org/10.1016/j.jiec.2008.05.006

Liu, Z., Li, L., Li, Z., & Tian, X. (2018). Removal of sulfate and heavy metals by sulfate-reducing bacteria in an expanded granular sludge bed reactor. *Environmental Technology (United Kingdom) 39*, 1814-1822. https://doi.org/10.1080/09593330.2017.1340347

Lohwacharin, J., & Annachhatre, A. P. (2010). Biological sulfide oxidation in an airlift bioreactor. *Bioresource Technology 101*, 2114-2120. https://doi.org/10.1016/j.biortech.2009.10.093

Lu, X., Zhen, G., Ni, J., Hojo, T., Kubota, K., & Li, Y. Y. (2016). Effect of influent COD/SO4 ratios on biodegradation behaviors of starch wastewater in an upflow anaerobic sludge blanket (UASB) reactor. *Bioresource Technology 214*, 175-183. https://doi.org/10.1016/j.biortech.2016.04.100

Lu, X., Zhen, G., Ni, J., Kubota, K., & Li, Y. Y. (2017). Sulfidogenesis process to strengthen re-granulation for biodegradation of methanolic wastewater and microorganisms evolution in an UASB reactor. *Water Research 108*, 137-150. https://doi.org/10.1016/j.watres.2016.10.073

Martins, M., Santos, E. S., Faleiro, M. L., Chaves, S., Tenreiro, R., Barros, R. J., Barreiros, A., & Costa, M. C. (2011). Performance and bacterial community shifts during bioremediation of acid mine drainage from two Portuguese mines. *International Biodeterioration and Biodegradation 65*, 972-981. https://doi.org/10.1016/j.ibiod.2011.07.006

Metcalf, E., & Eddy, H. (Eds.). (2014). *Wastewater Engineering: Treatment and Resource Recovery*. AECOM.

Meyer, D. D., de Andrade, P. A. M., Durrer, A., Andreote, F. D., Corção, G., & Brandelli, A. (2016). Bacterial communities involved in sulfur transformations in wastewater treatment plants. *Applied Microbiology and Biotechnology 100*, 10125-10135. https://doi.org/10.1007/s00253-016-7839-3

O'Flaherty, V., Mahony, T., O'Kennedy, R., & Colleran, E. (1998). Effect of pH on growth kinetics and sulphide toxicity thresholds of a range of methanogenic, syntrophic and sulphate-reducing bacteria. *Process Biochemistry 33*, 555-569.

Ochoa-Herrera, V., León, G., Banihani, Q., Field, J. A., & Sierra-Alvarez, R. (2011). Toxicity of copper(II) ions to microorganisms in biological wastewater treatment systems. *The Science of the Total Environment 412-413*, 380-385. https://doi.org/10.1016/j.scitotenv.2011.09.072

Plascencia-Jatomea, R., González, I., Gómez, J., & Monroy, O. (2015). Operation and dynamic modeling of a novel integrated anaerobic-aerobic-anoxic reactor for sewage treatment. *Chemical Engineering Science 138*, 31-40. https://doi.org/10.1016/j.ces.2015.07.051

Pokorna-Krayzelova, L., Vejmelková, D., Selan, L., Jenicek, P., Volcke, E. I. P., & Bartacek, J. (2018). Final products and kinetics of biochemical and chemical sulfide oxidation under microaerobic conditions. *Water Science and Technology 78*, 1916-1924. https://doi.org/10.2166/wst.2018.485

Pokorna, D., & Zabranska, J. (2015). Sulfur-oxidizing bacteria in environmental technology. *Biotechnology Advances 33*, 1246-1259. https://doi.org/10.1016/j.biotechadv.2015.02.007

Sahinkaya, E., & Yucesoy, Z. (2010). Biotreatment of acidic zinc- and copper-containing wastewater using ethanol-fed sulfidogenic anaerobic baffled reactor. *Bioprocess and Biosystems Engineering 33*, 989-997. https://doi.org/10.1007/s00449-010-0423-9

Serrano, A., Peces, M., Astals, S., & Villa-Gómez, D. K. (2020). Batch assays for biological sulfate-reduction: a review towards a standardized protocol. *Critical Reviews in Environmental Science and Technology 50*, 1195-1223. https://doi.org/10.1080/10643389.2019.1644103

Shan, L., Zhang, Z., Yu, Y., Ambuchi, J. J., & Feng, Y. (2017). Performance of CSTR-EGSB-SBR system for treating sulfate-rich cellulosic ethanol wastewater and microbial community analysis. *Environmental Science and Pollution Research 24*, 14387-14395. https://doi.org/10.1007/s11356-017-9022-5

Show, K. Y., Yan, Y. G., Zhao, J., Shen, J., Han, Z. X., Yao, H. Y., & Lee, D. J. (2020). Laboratory trial and full-scale implementation of integrated anaerobic-aerobic treatment for high strength acrylic acid wastewater. *Science of the Total Environment, 738*, 140323. https://doi.org/10.1016/j.scitotenv.2020.140323

Sierra-Alvarez, R., Hollingsworth, J., & Zhou, M. S. (2007). Removal of copper in an integrated sulfate reducing bioreactor-crystallization reactor system. *Environmental Science and Technology 41*, 1426-1431. https://doi.org/10.1021/es062152l

Sim, M. S., Sessions, A. L., Orphan, V. J., & Adkins, J. F. (2019). Precise determination of equilibrium sulfur isotope effects during volatilization and deprotonation of dissolved H_2S. *Geochimica et Cosmochimica Acta 248*, 242-251. https://doi.org/10.1016/j.gca.2019.01.016

Sun, J., Dai, X., Liu, Y., Peng, L., & Ni, B. J. (2017). Sulfide removal and sulfur production in a membrane aerated biofilm reactor: Model evaluation. *Chemical Engineering Journal 309*, 454-462. https://doi.org/10.1016/j.cej.2016.09.146

Visser, A., Hulshoff Pol, L. W., & Lettinga, G. (1996). Competition of methanogenic and sulfidogenic bacteria. *Water Science and Technology 33*, 99-110. https://doi.org/10.1016/0273-1223(96)00324-1

Xingyu, L., Zou, G., Wang, X., Zou, L., Wen, J., Ruan, R., & Wang, D. (2013). A novel low pH sulfidogenic bioreactor using activated sludge as carbon source to treat acid mine drainage (AMD) and recovery metal sulfides: Pilot scale study. *Minerals Engineering 48*, 51-55. https://doi.org/10.1016/j.mineng.2012.11.004

Xu, X. J., Chen, C., Wang, A. J., Fang, N., Yuan, Y., Ren, N. Q., & Lee, D. J. (2012). Enhanced elementary sulfur recovery in integrated sulfate-reducing, sulfur-producing rector under micro-aerobic condition. *Bioresource Technology 116*, 517-521. https://doi.org/10.1016/j.biortech.2012.03.095

Yuan, Y., Chen, C., Zhao, Y., Wang, A., Sun, D., Huang, C., Liang, B., Tan, W., Xu, X., Zhou, X., Lee, D. J., & Ren, N.

(2015). Influence of COD/sulfate ratios on the integrated reactor system for simultaneous removal of carbon, sulfur and nitrogen. *Water Science and Technology 71*, 709-716. https://doi.org/10.2166/wst.2014.533

Zhang, W., Xiao, P., & Wang, D. (2014). Central treatment of different emulsion wastewaters by an integrated process of physicochemically enhanced ultrafiltration and anaerobic-aerobic biofilm reactor. *Bioresource Technology 159*, 150-156. https://doi.org/10.1016/j.biortech.2014.02.067

Zhao, J., Fang, D., Zhang, P., & Zhou, L. (2017). Long-term effects of increasing acidity on low-pH sulfate-reducing bioprocess and bacterial community. *Environmental Science and Pollution Research 24*, 4067-4076. https://doi.org/10.1007/s11356-016-8147-2

Zhou, J., & Xing, J. (2015). Effect of electron donors on the performance of haloalkaliphilic sulfate-reducing bioreactors for flue gas treatment and microbial degradation patterns related to sulfate reduction of different electron donors. *Biochemical Engineering Journal 96*, 14-22. https://doi.org/10.1016/j.bej.2014.12.015