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Sulfidogenic activity related to microbial diversity in a biological system employed for sulfate-rich wastewater treatment

Actividad sulfidogénica relacionada con la diversidad microbiana en un sistema de tratamiento biológico para aguas ricas en sulfato

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Abstract
Industrial activity disrupts the natural sulfur cycle by the generation of sulfate-rich wastewater. Nonetheless, these effluents can be treated in anaerobic reactors. This study seeks to relate sulfidogenic activity with microbial diversity during the start-up of an anaerobic sulfate-reducing reactor. An anaerobic sludge was characterized employing 16s rRNA assays. The sludge was adapted to sulfate-reducing activity in an up-flow fixed bed reactor with 2 and 1.5 COD/SO_4^{2-} ratios. Simultaneous organic matter removal and sulfate conversion to sulfide were analyzed. Finally, a kinetic study was conducted to determine sulfate conversion and sulfide production reaction rates. A first-order model was applied to determine the reaction rates for sulfate and sulfide. Microorganisms employed for bioremediation techniques, like *Pseudomonas azotoformans*, *Desulfovibrio*, and *Methanolinea* sp., were identified in the anaerobic sludge. The decrease in COD/SO_4^{2-} ratio did not negatively impact the overall efficiencies in the reactor in terms of COD and sulfate removal and sulfide production, reaching values of 76.54%, 81.53%, and 268.84 mgS^{2-}/L, respectively. Sulfidogenic activity was higher under higher sulfate concentrations (%H_2S-COD = 55.2 for a 1.5 COD/SO_4^{2-} ratio). The results indicate a gradual increase in sulfidogenic activity; therefore, sulfate-reducing conditions were attained in the system.
Keywords: sulfate-reduction, sulfidogenic activity, sulfate-rich wastewater, microbial diversity, reaction rates.

Resumen
La generación de efluentes ricos en sulfatos puede causar afectaciones en el ciclo del azufre; sin embargo, dichos efluentes pueden ser tratados en reactores anaerobios. Este estudio busca relacionar la actividad sulfidogénica con la diversidad microbiana durante la adaptación de un reactor anaerobio sulfato-reductor. Un inóculo anaerobio fue caracterizado con base en técnicas moleculares. Posteriormente, el inóculo fue adaptado a condiciones sulfato-reductoras en un reactor de lecho fijo con relaciones de 2 y 1.5 DQO/SO_4^{2-}. Simultáneamente se analizó la remoción de materia orgánica y la conversión de sulfato a sulfuro. Finalmente, se realizó un estudio cinético para determinar las velocidades de reacción para la conversión de sulfatos y producción de sulfuro. La diversidad microbiana del consorcio empleado permitió la identificación de microrganismos empleados para técnicas de remediación biológica, como *Pseudomonas azotoformans*, *Desulfovibrio* y *Methanolinea* sp. La disminución en la relación de DQO/SO_4^{2-} no afectó las eficiencias globales del reactor, en términos de remoción de materia orgánica y sulfatos, así como la producción de sulfuros, alcanzando valores de 76.54%, 81.53% y 268.84 mgS^{2-}/L, respectivamente. La actividad sulfidogénica fue mayor a concentraciones elevadas de sulfatos (%H_2S-COD = 55.2 para una relación de 1.5 COD/SO_4^{2-}). Los resultados indican un incremento gradual en la actividad sulfidogénica, por lo que se alcanzaron condiciones sulfato-reductoras en el sistema.
Palabras clave: sulfato reducción, actividad sulfidogénica, aguas contaminadas con sulfato, diversidad microbiana, velocidad de reacción.

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1 Introduction

Industrial activity, like petroleum refining, paper manufacturing, pharmaceutics, desalination, and food processing, disrupts the natural sulfur cycle (Ding & Zeng, 2022; Guo *et al*., 2019). Rubber skim wastewater and acid mine drainage (AMD) are some examples of sulfate-rich wastewater (Paepatung *et al*., 2020; Park *et al*., 2019). Under anaerobic conditions, sulfate-reducing bacteria (SRB) can use sulfate as an electron acceptor and reduce it to sulfides, which can precipitate metals in sulfate-rich environments (Hu *et al*., 2020). SRB are the vital functional microorganisms that regulate a sulfidogenic bioprocess; some examples are *Desulfobacter*, *Desulfococus*, *Desufosarcina*, *Desulfovibrio*, *Dsulfomicrobium*, and *Desulfobotulus* (Huang *et al*., 2020).

Bioremediation studies concerning wastewater treatment have established pilot/full-scale remediation technologies, like anaerobic bioreactors (Yin *et al*., 2019). Anaerobic digestion can treat many effluents and is achieved by complex microbial communities (Vigueras-Carmona *et al*., 2022). Some advantages of anaerobic digestion employed for sustainable wastewater management include low sludge yield, low nutrient requirement, low energy demand, high organic loading rates, and biogas production (Maleki *et al*., 2018).

Anaerobic digestion is a multistage process that includes hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Vera-Perez *et al*., 2023). Microorganisms break down substrates in an oxygen-free environment, producing biogas, comprising methane, carbon dioxide, hydrogen sulfide, ammonia, hydrogen, and water vapor (Kunatsa & Xia, 2022). During anaerobic digestion, bacteria are responsible for hydrolysis, acidogenesis, and acetogenesis, while archaea conduct methanogenesis. Archaea and syntrophic bacteria, specifically SRB, compete for complex substrates within the reactors (Damodara Kannan *et al*., 2020). Some parameters that affect the efficiency of sulfate-reducing reactors include hydraulic retention time (HRT), temperature, pH, COD/SO_4^{2-} ratios, as well as the inoculum (Habe *et al*., 2020; Vera-Perez *et al*., 2023). Apart from these parameters, understanding population dynamics and interactions is essential to maintain steady-state anaerobic digestion operations.

Understanding biodegradation mechanisms, predicting digestor performance, and designing anaerobic processes is possible using kinetic studies (Sajeena Beevi *et al*., 2015). Nonetheless, only a few studies focus on the kinetic side of anaerobic digestion, specifically for sulfate reduction. Most studies focus on logistic models like Monod and Gompertz or consider simple organic compounds as substrates (Bayu *et al*., 2022). Additionally, kinetic models like zero, first, pseudo-first, second, or third-order reaction models can be employed to evaluate substrate reduction in bioreactors (Kumar *et al*., 2020). Finally, kinetic models can be used as a scale-up tool, process optimization, and prediction of the performance of anaerobic processes (Hassan *et al*., 2022). Continuous systems allow a continuous substrate flow in and out of the system, allowing growth requirements for microorganisms to be constant over time. Continuous systems reach a steady state based on their outlet conditions; therefore, they can be used for kinetic measurements (Maleki *et al*. 2018).

Although sulfate reduction has been widely studied, little focus has been paid to correlating microbial diversity and kinetics of sulfate conversion in bioreactors. Therefore, this study seeks to evaluate the relationship between sulfidogenic activity and initial microbial diversity during the start-up operation of an anaerobic sulfate-reducing reactor. Simultaneous organic matter removal and sulfate conversion to sulfide were analyzed. Finally, a kinetic study for sulfate conversion reaction rates was conducted.

2 Materials and methods

2.1 Sludge characterization

An anaerobic sludge collected from a wastewater treatment plant (WWTP) with a VS/TS ratio of 51.01 ± 0.67 % was employed for this study. The microbial diversity of the anaerobic sludge was characterized employing 16s rRNA assays.

DNA isolation was performed on a sample of anaerobic sludge using an Invitrogen^{TM} PureLink® Genomic DNA Mini Kit (Catalog No. K1820-01, K1820-02, K182-04), following the guidebook (Invitrogen^{TM}, n.d.). The process consisted of three stages: lysis, precipitation, and purification/elution. The presence of DNA was verified with a 1% agarose gel electrophoresis with ethidium bromide.

DNA samples were analyzed in the "Research and Testing Laboratory" in Texas, USA. The universal primers used for PCR reactions for bacteria and archaea are shown in Table 1, followed by the metagenomic sequencing platform Illumina MiSeq®. The multiple sequences were aligned with *FastTree* (Price *et al*., 2010). The Krona software was used to analyze the abundance of taxonomic genes.

Table 1. Primers employed for metagenomic DNA amplification of the anaerobic sludge.

Primer		Essay	Diversity
Arch517F - GCYTAAAGSRNCCGTAGC	Arch909R TTTCAGYCTTGCGRCCGTAC	Arch909R	Archaea
28F -GAGTTTGATCNTGGCTCAG	519R GTNTTACNGCGGCKGCTG	28F	Bacteria

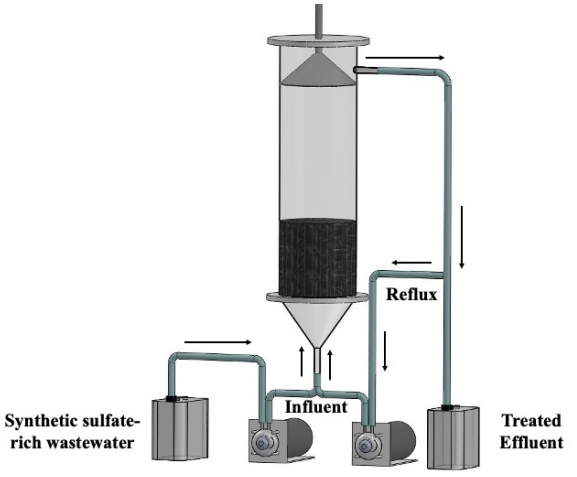

Figure 1. Schematic representation of the anaerobic sulfate-reducing reactor.

2.2 Reactor adaptation to sulfate-reducing conditions

The anaerobic sludge was adapted to sulfate-reducing conditions in an up-flow fixed bed reactor. The sulfate-reducing reactor (Figure 1) consisted of a glass column with a 5.93 cm internal diameter, 27.62 cm height, and 850 mL operating volume. The reactor was inoculated with 30 - 40 % liquified sludge and deactivated zeolite as biomass support (particle size 2 mm and Si/Al ratio of 4.53).

Table 2 shows the operational conditions maintained in the reactor. The reactor operated in a controlled temperature room at 35 °C, using automatic heaters. The reactor operated with a 2 g COD/g SO_4^{2-} ratio; once the system reached a steady state, the ratio was lowered to 1.5. COD was maintained at 2 g COD/L, glucose was used as a carbon source, and sulfate was added using sodium sulfate (1 and 1.33 g SO_4^{2-}/L). The culture media used in the reactor contained (mg/L): NH_4Cl (1045), KH_2PO_4 (170), $MgSO_4{\cdot}7H_2O$ (185), $CaCl_2{\cdot}H_2O$ (50), $NaHCO_3$ (2000), yeast extract (20), and 1 mL/L of trace element solution. The trace element solution contained (mg/L): $FeCl_2{\cdot}4H_2O$ (2000), $MnCl_2$ (500), EDTA·$2H_2O$ (500), H_3BO_3 (50), $AlCl_3$ (50), $NiCl_3{\cdot}6H_2O$ (50), $CoCl_2{\cdot}6H_2O$ (50), Na_2SeO_3 (100), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (50), $CuCl_2{\cdot}2H_2O$ (50), resazurin (200), and 36% HCl (1 mL/L) (Visser *et al*., 1996).

Table 2. Operational parameters of the anaerobic sulfate-reducing reactor.

COD/SO_4^{2-} (g/g)	2	1.5
Volume (mL)	850	850
pH	7 - 8	7 - 8
OLR (gCOD/L·d)	2	2
SLR (gSO_4^{2-}/L·d)	1	1.33
Temperature (°C)	35	35
HRT (h)	24	24

Daily pH, temperature, alkalinity, COD, sulfate, and sulfide concentrations were determined in the inlet and outlet of the reactor. Considering liquid and gas phases, total sulfide concentration was determined using equation 7. Methane production was not determined.

2.3 Calculations

2.3.1 Percentage of electron equivalents and efficiencies

Percentage of electron equivalents, COD and sulfate removal, and sulfide generation efficiencies were determined following the procedure described in a previous study (Loreto-Muñoz *et al*., 2021).

Electron equivalents indicate the electron flow towards sulfidogenic activity based on COD removal rates, sulfide stoichiometric yield, and sulfide generation. The percentage of electron equivalents towards sulfidogenic activity was determined using equation 1:

$$\%H_2S - COD = 100 \times \frac{S \cdot F_s}{COD_R} \tag{1}$$

where: S = total sulfide in liquid and gas phase yield (g S^{2-}/L·d); CODR = organic matter removal rate (g COD/L·d), and Fs = 2 g S^{2-}-COD/gS^{2-}.

The COD (%COD_R, Eq. 2) and sulfate removal (%SO_4^{2-}, Eq. 3) efficiencies were calculated as follows:

$$\%COD_R = 100 \times \frac{CO_I - COD_E}{COD_I} \tag{2}$$

$$\%SO_4^{2-}{}_R = 100 \times \frac{SO_4^{2-}{}_I - SO_4^{2-}{}_E}{SO_4^{2-}{}_I} \tag{3}$$

where: COD_I and COD_E = inlet and effluent COD, $SO_4^{2-}{}_I$ and $SO_4^{2-}{}_E$ = inlet and outlet sulfate concentrations in the reactor.

The total sulfide generation efficiency was determined using the theoretical sulfide concentration and stoichiometry as follows:

$$S^{2-}_{Theoretical} = \frac{11 \times (SO_4^{2-}{}_I - SO_4^{2-}{}_E)}{96} \quad (4)$$

$$\%S^{2-} = 100 \times \frac{S^{2-}_{L+G}}{S^{2-}_{Theoretical}} \quad (5)$$

where: $\%S^{2-}$ = total sulfide generation efficiency, S^{2-}_{L+G} = total sulfide generation in liquid and gas phase, and $S^{2-}_{Theoretical}$ = theoretical sulfide generation based on sulfate removal.

2.3.2 *Total sulfide*

Total sulfide was determined using Henry´s Law for gas-liquid equilibrium and dissociation constants, reaching the following correlation:

$$S^{2-}_{L+G} = [H_2S]_l + [H_2S]_g \quad (6)$$

Where:

$$S^{2-}_{L+G} = [H_2S]_l + \frac{[H_2S]_l}{(1 + K_{H_2S}\, 10^{pH})a} \quad (7)$$

$$K_{H_2S} = (0.382T + 1.892)10^{-8} \quad (8)$$

$$a = 3.442 - 0.044T \quad (9)$$

Where: α = equilibrium constant; K_{H_2S} = Henry´s constant; S^{2-}_{L+G} = total sulfide in liquid and gas phase; $[H_2\ S]_l$ = sulfide concentration in the effluent in the liquid phase (mg/L); T = temperature (°C).

2.3.3 *Kinetic study*

The reaction rates of sulfate and sulfide and reaction rate constants were determined based on design equations for chemical reactors and first-order kinetic models. The latter compares stable process performance under practical conditions and is usually applied to anaerobic digestion of complex substrates (Sajeena Beevi *et al*. 2015):

$$\frac{dC_i}{dt} = -kC_i \quad (10)$$

Where: C_i = species concentration in the reactor (g/L); and k = reaction rate constant (d^{-1}).

Anaerobic digestion is a complex process, so several assumptions need to be made. The employed system is a tubular reactor with reflux and constant volume. The volumetric flows in the inlet and outlet of the reactor were equal and remained constant. No distinction between biomass and chemical reactions was made. Given that the reflux is fifteen times higher than the volumetric flow, the design equation for a continuous stirred tank reactor (CSTR) can be employed:

$$(C_{i,i} - C_{o,i}) = -r\left(\frac{V}{Q}\right) \quad (11)$$

$$r = -\frac{C_{i,i} - C_{o,i}}{V/Q} \quad (12)$$

Where: C_i = species concentration in the inlet and outlet of the reactor (g/L); V = volume (L); Q = volumetric flow (L/d); and r = reaction rate (g/L·d).

2.4 *Analytical methods*

COD was determined using a HACH kit, with samples being aerated for 2 min and centrifuged at 6500 rpm for 15 min to ensure that sulfide compounds volatize or oxidize. Sulfate was determined by the turbidimetric method, and sulfide (in the liquid phase) by the iodometric method (American Public Health Association, 2012). COD, sulfates, and sulfides were determined as triplicates (n = 3) for accuracy. A HANNA instruments potentiometer (HANNA-HI5522) was employed for pH measurement.

3 Results and discussion

3.1 *Sludge characterization*

Figures 2 and 3 show the Krona charts for the identified bacteria and archaea obtained from the 16s rRNA assay of the sludge sample. A total of 38 bacteria and 7 archaea were identified. The software Krona allows the representation of the taxonomic classification analysis of microbial communities based on family, genus, order class, and phylum in a single multilevel species-taxonomy diagram (Upadhyay *et al*., 2023). Domain-to-order levels are presented in the internal to external circles of the chart, allowing rapid analysis of the metagenome samples (Le Zhang *et al*., 2019).

Within the bacteria domain, phylum *Proteobacteria*, *Chloroflexi*, and *Nitrospirae* stood out; among the phylum *Proteobacteria*, the orders *Pseudomonadales*, *Enterobacteriales*, *Desulfuromonadales*, *Desulfobacterales*, and *Syntrophobacterales* were found. The dominant bacteria in the anaerobic sludge was *Pseudomonas azotoformans*, with a relative abundance of 38%.

Some of the identified microorganisms have different applications in remediation technologies. For example, *Pseudomonas azotoformans* can fix nitrogen, degrade complex molecules, and produce exopolysaccharides (Velázquez *et al*., 2016).

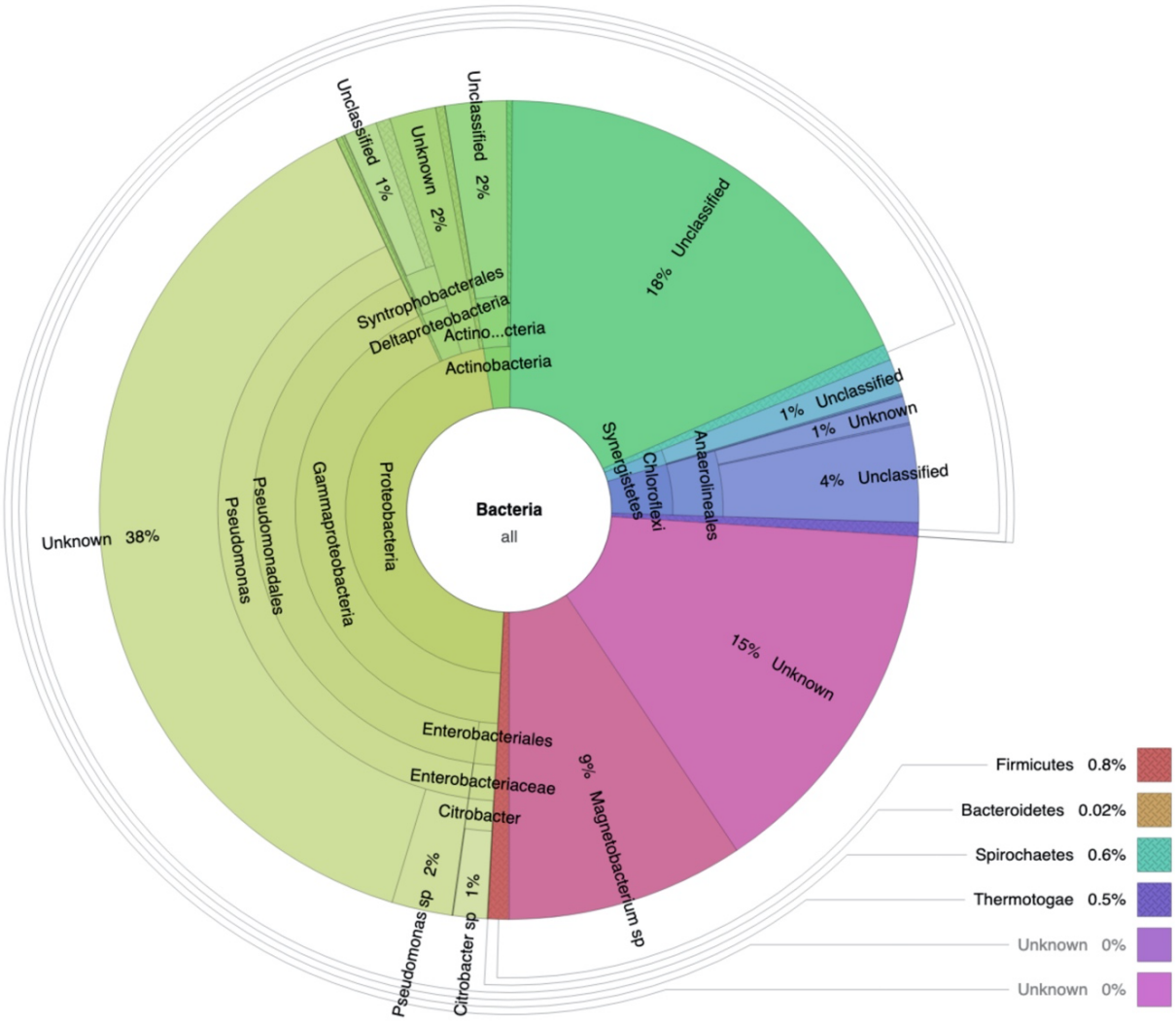

Figure 2. Krona chart of multilevel taxonomy of the microbial community of bacteria in the anaerobic sludge.

Found in a lower abundance, *Trichococcus* sp. (0.01 %) can decompose complex organic matter like lactate to acetate, which reduces sulfate to sulfite. Also, it presents tolerance to Hg (II) and Pb (II) (Liang Zhang *et al*., 2016). Additionally, sulfate-reducing microorganisms from the genus Citrobacter sp., *Desulfovibrio*, *Desulfobacterales*, and *Desulfobullbus propionicus* were found in lower proportions.

Only the phylum *Crenarchaeota* and *Euryarchaeota* were identified within the archaea domain. Among the phylum *Euryarchaeota* the *Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales* orders were found (Figure 3). Some of the identified archaea found in the anaerobic sludge include *Methanobacterium* sp. (55%), *Methanolinea* sp. (24%), and *Methanbacterium beijingense* (15%). *Methanobacterium* sp. can convert hydrogen into methane, while *Methanbacterium beijingense* uses hydrogen and CO_2 for methanogenesis. Both are commonly found in methanogenic bioreactors (Celis *et al*., 2009).

3.2 *Reactor adaptation to sulfate-reducing conditions*

Figure 4 and Table 3 show the overall efficiencies obtained during the operation of the anaerobic sulfate-reducing reactor. The total operational time of the reactor was 103 d. The reactor operated in two phases, variating COD/SO_4^{2-} ratios (2 and 1.5).

The increase in sulfate concentration in the inlet of the reactor did not negatively affect the overall efficiencies in the reactor. Sulfate removal remained constant, and COD removal increased with the reduction in COD/SO_4^{2-} ratios or sulfate increase (Table 3). Nonetheless, total sulfide production increased from 170 to 268.8 mg S^{2-}/L but decreased in the gaseous phase, indicating increased sulfate conversion to sulfide. Although sulfides can cause an inhibitory effect on anaerobic digestion or process

instability, COD and sulfate removal efficiencies indicate that the produced sulfide (170 - 270 mg S^{2-}/L) does not cause a significant product inhibition in the consortium (De La Cueva *et al*., 2021).

Methane production was not determined, but the microbial diversity found in the sludge includes bacteria and archaea, like *Citrobacter* sp., *Desulfobulbus propionicus*, *Clostridium* sp., *Methanosaeta* sp., *Methanobacterium* sp., *Anaerolinea* sp., *Methanothermobacter*, and *Bacillus*, which have been employed for bioremediation of contaminated wastewater in anaerobic reactors (Bayu *et al*., 2022; Dafale *et al*., 2010; Deng *et al*., 2016; Giordani *et al*., 2019; Xu & Chen, 2020; Yuan *et al*., 2015). Syntrophic interactions between fermentative bacteria and methanogens are responsible for organic substrate degradation (Damodara Kannan *et al*., 2020). Therefore, the high COD removal can be attributed to other microorganisms in the consortium, not only sulfate-reducing bacteria, agreeing with the electron equivalents balance. From the total COD removal in the reactor, an average of 24.6% (2 g COD/g SO_4^{2-} ratio) and 34.5% (1.5 g COD/g SO_4^{2-} ratio) of the metabolic activity in the reactor was sulfidogenic. The remaining COD removal can be attributed to other metabolic processes like methanogenesis or biomass growth. Finally, the low abundance of sulfate-reducing microorganisms (Figure 2) can account for the low sulfidogenic activity in the system. Nonetheless, a gradual increase in sulfidogenic activity is observed in Figure 5, reaching peaks of 39.8% (2 g COD/g SO_4^{2-} ratio) and 55.2% (1.5 g COD/g SO_4^{2-} ratio), respectively. Therefore, the consortium can adapt to higher sulfate concentrations, increasing sulfidogenic activity with time.

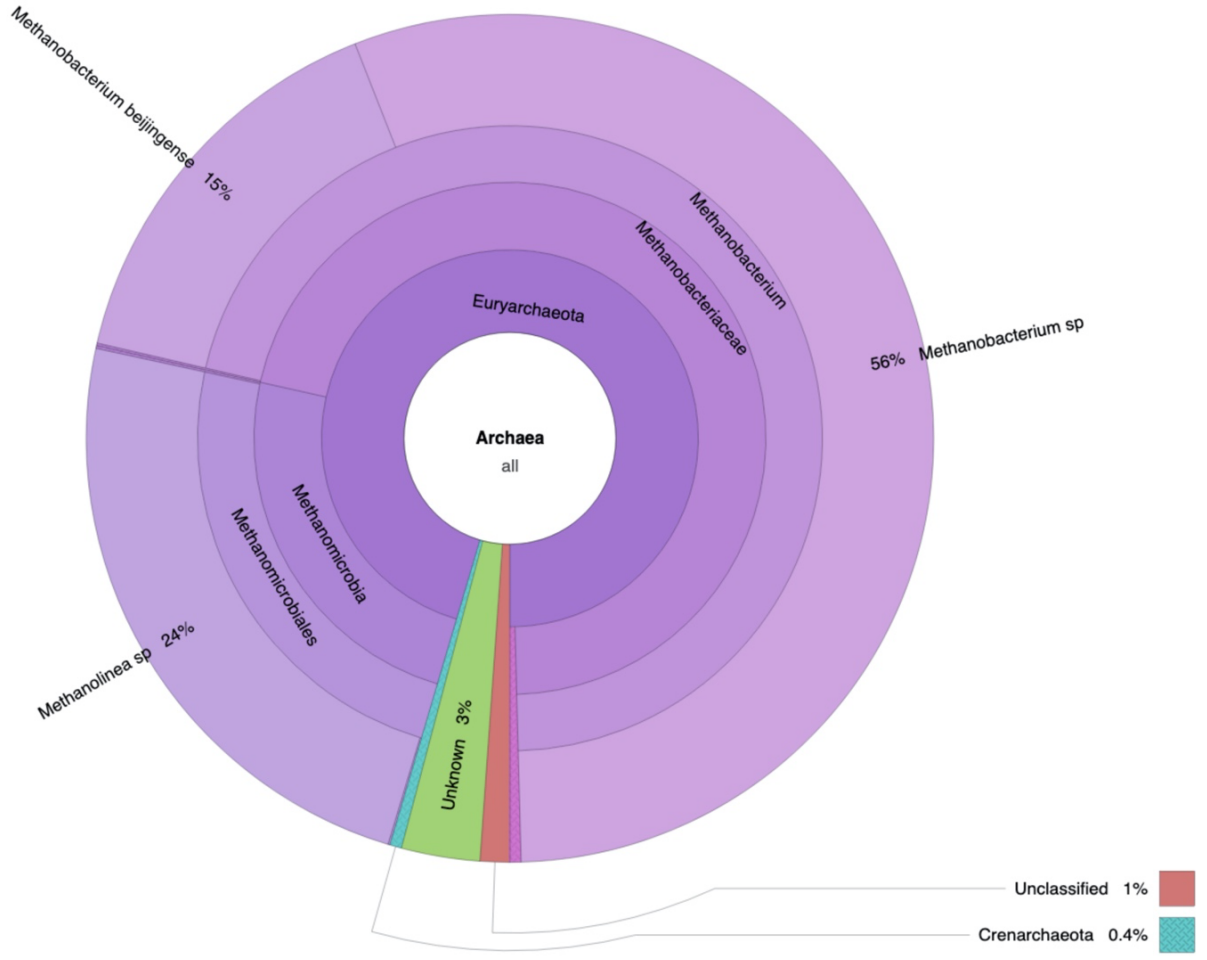

Figure 3. Krona chart of multilevel taxonomy of the microbial community of archaea in the anaerobic sludge.

Table 3. Average COD, sulfate, and sulfide efficiencies in the anaerobic reactor.

COD/SO_4^{2-} ratio	(g/g)	2	1.5
Time	(d)	44	59
pH	Influent	-	8.0
	Effluent	-	7.94 ± 0.23
	Alkalinity	-	0.433 ± 0.01
COD removal	(mg/L)	1293.98 ± 380.8	1574.96 ± 137.3
	(%)	63.81 ± 19.22	76.54 ± 5.46
SO_4^{2-} removal	(mg/L)	897.91 ± 462.7	1086.46 ± 172.1
	(%)	81.95 ± 14.306	81.53 ± 12.53
Sulfide production	Liquid (mg/L)	111.4 ± 15.05	177.3 ± 52.84
	Gas (mg/L)	58.58	27.78
	Liquid + Gas (mg/L)	170.0 ± 22.9	268.84 ± 81.9
	Efficiency* (%)	63.81	68.91
Percentage of electron equivalents*	$\%H_2S$ - COD Average	24.6	34.5
	$\%H_2S$ - COD Maximum	39.8	55.2

*Based on total sulfide production in liquid and gas phases.

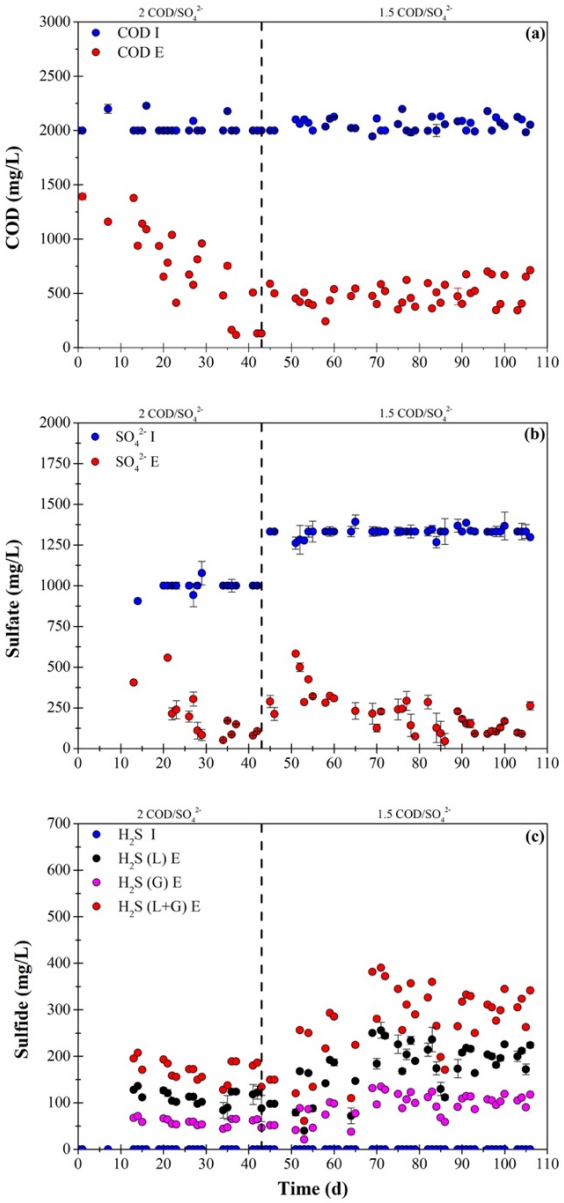

Figure 4. (a) COD; (b) sulfate; (c) sulfide (L = Liquid, G = Gas, and L+G = Liquid + Gas) concentrations in the influent and effluent of the anaerobic sulfate-reducing reactor as a function of time, for 2 and 1.5 g COD/g SO_4^{2-} ratios.

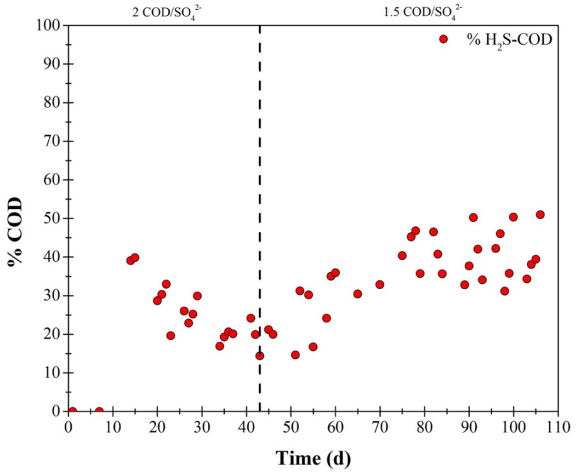

Figure 5. Electron equivalents for sulfidogenic activity as a function of time for 2 and 1.5 g COD/g SO_4^{2-} ratios.

3.3 Kinetic study

Sulfate concentration in the reactor outlet decays exponentially, while sulfide increases with time (Figure 4). Therefore, a first-order model was applied to verify the reaction kinetics order. The plot of reaction rate against species concentration adjusts to a first-order model with a regression coefficient equal to 1 for both species (Figure 6). Therefore, the reaction rate depends solely on sulfate and sulfide concentration. The reaction rate constant can be determined using the magnitude of the slope from Figure 6. Based on the attained reaction rate constant, the reaction rate is as follows:

$$r_{SO_4^{2-}} = -k[SO_4^{2-}] \quad (13)$$

$$r_{S^{2-}} = k[S^{2-}] \quad (14)$$

The magnitudes of the reaction rate constants remain constant with the decrease in g COD/g SO_4^{2-} ratio for both sulfate removal and sulfide production, which can indicate that it is independent of sulfate concentration (Table 4).

Table 4. Reaction rates and constants for sulfate and sulfide in the anaerobic reactor.

COD/SO_4^{2-}	g/g	2	1.5
$r_{SO_4^{2-}}$	mmol/L·d	-9.4 ± 0.54	-12.5 ± 0.80
$r_{S^{2-}}$	mmol/L·d	5.1 ± 0.77	10.0 ± 0.71
$k_{SO_4^{2-}}$	d^{-1}	0.00100	0.00100
$k_{S^{2-}}$	d^{-1}	0.00153	0.00153

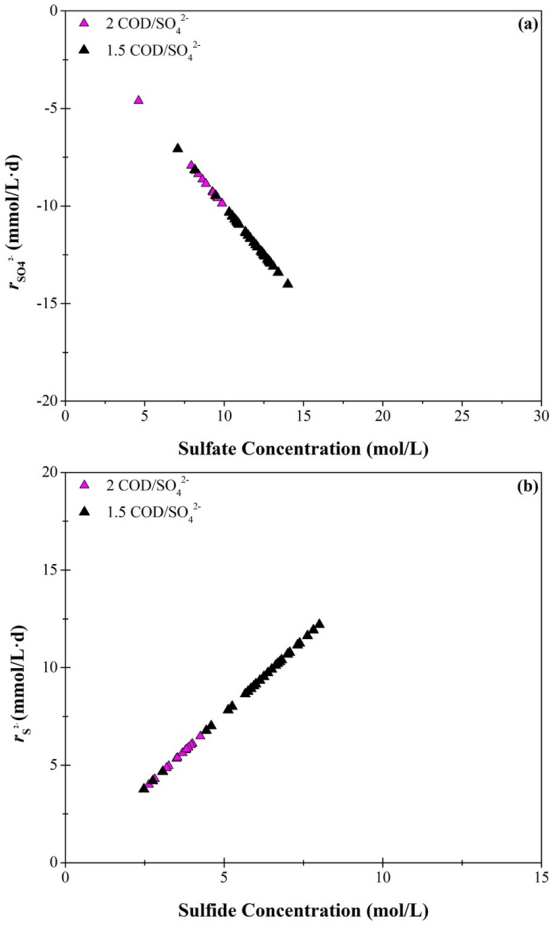

Figure 6. (a) Sulfate consumption; (b) sulfide production reaction rates as a function of the species concentration in the reactor for 2 and 1.5 g COD/g SO_4^{2-} ratios.

Additionally, Figure 6 shows that sulfate removal and sulfide production reaction rates are proportional. The reaction rates in Table 4 were determined as a mean value once the transition phase ended and the system reached a steady state. For both species, the average reaction rates were significantly higher for a 1.5 g COD/g SO_4^{2-}; this can be attributed to an adaptation to higher sulfate concentrations (inlet).

Conclusions

Identifying bacteria and archaea diversity was possible using second-generation massive sequencing. *Pseudomonas azotoformans* (38%) and Methanobacterium sp. (55%) were found in higher proportions. Nonetheless, sulfate-reducing microorganisms (*Desulfovibrio*, *Desulfobacter*ales, and Desulfobullbus propionicus), methane producers (Methanobacterium beijngense), and metal tolerant (Trichococcus sp.) were found in less proportion.

The decrease in COD/SO_4^{2-} ratios did not negatively impact the overall efficiencies of the system. However, an increase in sulfate-reducing activity was observed, and based on total sulfide production, sulfidogenic activity increased, reaching a peak of 55%. Other microorganisms present in the sludge can account for the remanent metabolic activity.

Kinetic studies show that a first-order kinetic model describes the reaction in the reactor. Both sulfide production and sulfate removal constants were significantly higher for a 1.5 COD/SO_4^{2-} ratio.

Acknowledgment

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Nomenclature

AMD	Acid mine drainage
SRB	Sulfate-reducing bacteria
UASB	Up-flow anaerobic sludge bed reactor
CSTR	Continuous stirred tank reactor
WWTP	Wastewater treatment plant
OLR	Organic loading rate
SLR	Sulfate loading rate
VS	Volatile solids
TS	Total solids
COD	Chemical oxygen demand
SO_4^{2-}	Sulfate
S^{2-}	Sulfide
$[H_2S]_{(g)}$	Sulfide concentration in gaseous phase
$[H_2S]_{(l)}$	Sulfide concentration in liquid phase
α	Henry´s constant
KH_2S	Dissociation constant
$\%H_2S$-COD	Percentage of electron equivalents towards sulfidogenic activity

S	Total sulfide in liquid and gas phase yield
COD_R	Organic matter removal rate
Fs	Sulfide stoichiometric ratio
$\%COD_R$	Chemical oxygen demand removal efficiency
COD_I	Chemical oxygen demand concentration in the inlet
COD_R	Chemical oxygen demand concentration in the effluent
L	Liquid phase
G	Gas phase
L + G	Liquid plus gas phase
$\%\ SO_4^{2-}{}_R$	Sulfate removal efficiency
$SO_4^{2-}{}_I$	Sulfate concentration in the inlet
$SO_4^{2-}{}_E$	Sulfate concentration in the effluent
$\%S^{2-}$	Total sulfide generation efficiency
$S^{2-}_{Theoretical}$	Theoretical sulfide generation
S^{2-}_{L+G}	Total sulfide in liquid and gas phase
I	Influent
E	Effluent
Ci,i	Species concentration in the inlet
Ci,o	Species concentration in the outlet
HRT	Hydraulic retention time
V	Volume
Q	Volumetric flow
r_i	Reaction rate
k_i	Reaction rate constant

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