Revista Mexicana de Ingeniería Química

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

Reducción de sulfato en un lodo aclimatado gradualmente a acetato como único donador de electrones y su potencial aplicación como inóculo en una celda de combustible microbiana

J.R. González-Paz[1], A. Ordaz[2], J. Jan-Roblero[3*], L.C. Fernández-Linares[1] and C. Guerrero-Barajas[1*]

[1]*Laboratorio de Biotecnología Ambiental, Departamento de Bioprocesos, Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional, Av. Acueducto s/n, Col. Barrio la Laguna Ticomán, Ciudad de México, 07340, México.*
[2]*Laboratorio de Bioingeniería Ambiental, Unidad de Estudios Superiores de Tultitlán, Universidad Mexiquense del Bicentenario, Av. Ex-Hacienda de Portales s/n Villa Esmeralda, Tultitlán de Mariano Escobedo, Estado de México, 54910, México.*
[3]*Laboratorio de Biotecnología Ambiental. Departamento de Microbiología. Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional. Prol. de Carpio y Plan de Ayala s/n, Col. Santo Tomás, Ciudad de México, 11340, México.*

Abstract

The aim of the present work was to obtain a sulfidogenic sludge capable of thriving with acetate and evaluate its potential application as a source of microorganisms in a microbial fuel cell (MFC). The results showed that sulfate (SO_4^{-2}) to sulfide (HS^-) conversion increased from 74 ± 0.39% with a fed consisting of a mixture acetate:butyrate, to 85 ± 0.34% with a fed containing only acetate. The sulfate removal rate (SRR) was of 1755.30 ± 50 mg SO_4^{-2} gVSS^{-1} L^{-1} d^{-1} and sulfate reducing activity (SRA) 155.21 ± 30 mg COD-H_2S gVSS^{-1} d^{-1} with the mixture and SRR 1692.31 ± 39 mg SO_4^{-2} gVSS^{-1} L^{-1} d^{-1} ; SRA 184.25 ± 24 mg COD-H_2S gVSS^{-1} d^{-1} with acetate. The COD removal increased from 66 ± 0.8% with the mixture to 81 ± 0.39%, with acetate. The open circuit voltage increased from 0.668 to 0.788 V with acetate. The results suggested that this sludge could be utilized to recover energy that could be eventually susceptible to storage for further utilization while reducing sulfate and avoiding accumulation of acetate.

Keywords: Sulfate reduction, acetate, microbial fuel cell.

Resumen

El objetivo del presente trabajo fue obtener un lodo sulfurogénico capaz de utilizar acetato y evaluar su potencial aplicación como fuente de microorganismos en una celda de combustible microbiana (CCM). Los resultados mostraron que la conversión de sulfato (SO_4^{-2}) a sulfuro (HS^-) se incrementó de 74 ± 0.39% con una alimentación de acetato:butirato a 85 ± 0.34% con una alimentación que contenía solamente acetato. La velocidad de remoción de sulfato (VRS) fue de of 1755.30 ± 50 mg SO_4^{-2} g SSV^{-1} L^{-1} d^{-1} y la actividad sulfato reductora (ASR) fue de 155.21 ± 30 mg DQO-H_2S g SSV^{-1} d^{-1} con la mezcla, y la VRS fue de 1692.31 ± 39 mg SO_4^{-2} g SSV^{-1} L^{-1} d^{-1} con una ASR de 184.25 ± 24 mg DQO-H_2S gSSV^{-1} d^{-1} con solo acetato. La remoción de DQO se incrementó de 66 ± 0.8% con la mezcla a 81 ± 0.39%, con acetato solamente. El voltaje a circuito abierto se incrementó de 0.669 a 0.788 V con acetato. Los resultados sugieren que este lodo podría utilizarse para recuperar energía que eventualmente será susceptible de almacenamiento para su posterior utilización mientras se reduce sulfato y se evita la acumulación de acetato.

Palabras clave: Reducción de sulfato, acetato, celda de combustible microbiana.

1 Introduction

Microbial fuel cells (MFC) have been reported as an efficient alternative to generate electricity during the removal of COD in wastewater treatment (Logan *et al*., 2006; van den Brand *et al*., 2015; He *et al*., 2017).

This technology has been reported to require a low energetic cost, a lower sludge generation and also to be a process in which the removal of certain organic pollutants can be achieved (He *et al*., 2017; Sivasankar *et al*., 2018). Several types of wastewater effluents have been treated in MFC yielding electrical energy at different extent.

Some examples of these MFC have treated molasses waste streams mixed with sewage, obtaining 81% COD removal, a voltage of 762 mV and power density of 382.5 mW m^{-2} (Sevda *et al*., 2013). The wastewater treatment mixed with acetate (Karra *et al*., 2013), the utilization of raw sludge in the anodic chamber (Mohd Yusoff *et al*., 2013) and municipal wastewater treatment (Feng *et al*., 2013) have also been reported in this type of process yielding COD removals in a range of 70 to 85%. The voltages reported in these cases range from 154 to 760 mV and current densities are in a range of 40 to 380 mW m^{-2} depending on the design of the cell.

In regard to the treatment of wastewater with high content of sulfate in MFC, this has been suggested because biogenic sulfide has been proven as an efficient redox mediator and can act as electron donor to the anode, which could result in an increase of energy generation (Logan *et al*., 2006; Lovley 2006; Angelov *et al*., 2013). A MFC can be utilized for the treatment of wastewater with high sulfate content despite the potential competition that may exist between sulfate and the electrode as electron acceptor in those cases in which there is presence of microorganisms that present high affinity to the electrode. In such a case, the energetic yield may be lower than the obtained in absence of sulfate, but ideal scenarios in which a combination of highly electrogenic microorganisms with zero competition of electron acceptors (i.e., absence of sulfate, nitrate, iron etc.) are difficult to establish in wastewater treatment. The study of MFC involving sulfate is of interest since sulfate rich wastewaters may derive from several types of industries such as alimentary, pharmaceutical, pulp paper manufacturing and acid mine drainage (AMD) just to mention a few (Kaksonen and Puhakka, 2007; Sanchez-Andrea *et al*., 2014). They are also the result of the utilization of seawater for toilette flushing (Hao *et al*., 2014), which generates domestic wastewater streams with high sulfate content.

The utilization of acetate in MFC has been widely reported due to its utilization as electron donor by many electrogenic strain of bacteria including some sulfate reducing bacteria (SRB) that are classified as complete oxidizers (Logan *et al*., 2006). It has also been demonstrated that the addition of acetate is a good strategy to stimulate COD removal and stability in MFC, particularly when the wastewater presents a relatively low COD concentration (Karra *et al*., 2013; Stager *et al*., 2017). On the other hand, accumulation of acetate may occur also under sulfate reducing conditions since the SRB that are classified as incomplete oxidizers utilize other volatile fatty acids first such as for example lactate, butyrate or propionate, which are common residuals from the degradation of organic matter. The accumulation of acetate may lead to a cell disruption due to the lower pH that the medium could reach (Sanchez-Andrea *et al*., 2014), which could damage the continuity of the process. Actual scenarios that could include the combination of sulfate and acetate in wastewater − other than a constructed facility for a MFC − are for example, constructed wetlands (CW) or sedimentary microbial fuel cell (SMFC), in which is convenient that sulfate reduction occurs coupled to acetate consumption. Acetate has been proved to be a good electron donor in wastewater treatment with different sources of microorganisms, i.e., raw sludge or mixtures of anaerobic sludge etc. (Karra *et al*., 2013; Stager *et al*., 2017; Do *et al*., 2018) in which members of several families of sulfate reducing bacteria (*Desulfobacter, Desulfobacterium, Desulfobacula* and *Desulfoccocus*) can be present, therefore, MFC systems based on sulfate reduction coupled to acetate are feasible. Although there are reports in which high current densities are obtained derived from COD removal in MFC, in the large majority of them the utilization of a selective membrane − to improve the proton or cation exchange from one chamber to another −, or a catalyst in the cathode to enhance the reaction is reported. The utilization of membranes in the cell and catalysts in the cathode implies a MFC of elevated cost besides the risk that implies the inclusion of compounds that could be toxic to the environment if accidentally released, such as the $Fe(CN)_6^{-3}$ ion, frequently utilized in the cathode (He *et al*., 2017). Alternatively, the utilization of a salt bridge that minimizes the cost and diminishes the release of toxic compounds ($Fe(CN)_6^{-3}$) has also been evaluated for the transferring of protons/cations reporting good results (Muralidharan *et al*., 2011; Sevda and Sreekrishnan, 2012).

In the case of MFC in which sulfate reduction is carried out, studies have focused on the improvement of sulfide oxidation in the anode to increase the generation of energy in the cells. For this purpose, some studies have been conducted with pure strain of bacteria such as the SRB *Desulfovibrio desulfuricans* and lactate as electron donor (Zhao *et al*., 2008). In that work the sulfide derived from sulfate reduction was oxidized in the cathode for which different materials were evaluated. In recent studies, consortia of bacteria have also been evaluated in the generation of electricity in dual chamber MFC with sulfate in the

anodic chamber. Some examples of the composition of these consortia are: *Clostridium, Desulfovibrio, Aeromonas* and *Tethathiobacter*, evaluated with landfill leachate and lactate (Kumar *et al*., 2017a) as electron donors. The authors reported that the oxidation of sulfide on the anode was attributed to *Aeromonas* and *Tethathiobacter*. Sulfur species such as sulfide, sulfur and thiosulfate were derived from sulfate reduction and acetate from the fermentation of lactate. Another consortium reported by these authors was composed of the SRB *Desulfovibrio aminophilus* combined with the sulfide oxidizing bacteria *Advenella kashmirensis* in the anodic chamber in which the oxidation of sulfide was improved (Kumar *et al*., 2017b). Reports on MFC for sulfate rich wastewater treatment with activated sludge and lactate as electron donor have been reported by Lee *et al*., (2012) obtaining sulfide, thiosulfate and sulfur. Most of the reports on MFC and sulfate include lactate as electron donor and little or null information on the performance of the cell in the presence of acetate.

Previous work in our research group has been conducted with a sulfidogenic sludge generated from hydrothermal vent sediments. This sludge has presented an absence of competition with methanogenic microorganisms − which are more sensitive to sulfide −, a high sulfate reducing activity (SRA) and a tolerance to relatively high concentrations of sulfide with mixtures of VFA that include acetate (García-Solares *et al*., 2014). The tolerance to high concentrations of sulfide and the acetate assimilation in a mixture of VFA suggest that the sludge could be a potential source of electrogenic microorganisms in a MFC using acetate as electron donor.

The aim of the present work was to obtain a sulfidogenic sludge that utilizes acetate as the only electron donor by means of a gradual acclimation. The initial source of carbon was composed of acetate and butyrate and butyrate was gradually removed until acetate was the sole source of carbon. The evaluation of the robustness of the sulfate reducing sludge and the generation of energy were conducted by analyzing the conversion of sulfate to sulfide in a dual chamber MFC in which the proton (and cation) transfer was conducted through a salt bridge.

2 Materials and methods

2.1 Sulfidogenic sludge and hydrothermal vent sediments

The initial sulfidogenic sludge (0.017 g VSS g^{-1} sludge) was obtained from a UASB reactor that has been fed with synthetic wastewater rich in sulfate (6 g L^{-1}) with acetate and butyrate (4 g COD L^{-1}) as the source of carbon (3.5:1 COD proportion) and a COD/SO$_4^{-2}$ ratio of 0.67 on a regular basis. The hydrothermal vent sediment (0.037 g VSS g^{-1} sediment) was collected from Nayarit, Mexico as indicated in Guerrero-Barajas and García-Peña (2010).

2.2 Culture medium

The culture medium utilized in the entire experimental work was as follows, composition in (g L^{-1}): CaCl$_2$ · 2H$_2$O, 0.02; MgCl·6H$_2$O, 0.166; NaCl, 2; NH$_4$Cl, 0.56; K$_2$HPO$_4$, 1.2; NaH$_2$PO$_4$ · 2H$_2$O, 1.6 and yeast extract, 0.04. Vitamins solution 10 mL L^{-1} and trace metals solution 2 mL L^{-1}. The trace metals solution composition was as follows (g L^{-1}): H$_3$BO$_3$, 0.05; FeSO4·7H2O, 2.8; ZnSO$_4$ · 7H$_2$O, 0.106; MnSO$_4$ · 7H$_2$O, 0.70; (NH$_4$)$_6$Mo$_7$O$_{24}$ · 4H$_2$O, 0.05; AlK(SO$_4$)·12H$_2$O, 0.175; Na$_3$Co(NO$_2$)$_6$ 3.4; NiSO$_4$ · 6H$_2$O, 0.026; CuSO$_4$ · 5H$_2$O, 0.175; EDTA, 1 and resazurin, 0.2. The vitamins solution composition was as follows (g L^{-1}): biotin, 0.02; folic acid dehydrate, 0.02; pantothenic acid, 0.05; nicotinamide, 0.05; p-aminobenzoate, 0.05; thiamine, 0.05; lipoic acid, 0.05 and piridoxine, 0.1.

2.3 Microbial fuel cell inoculum preparation under sulfate reducing conditions

A mixture of the sulfidogenic sludge (0.017 g VSS g -1sludge) (66.6%) and hydrothermal vent sediments (0.037 g VSS g -1sediment) characterized by Guerrero-Barajas and García-Peña (2010) (33.3%) was prepared to a final wet weight of 52.5 g. This mixture was incubated for two weeks at room temperature (18-22 °C) in a serum flask with 500 mL of culture medium described in the section 2.2 and 6 g L^{-1} of sulfate (SO$_4^{-2}$) as sodium sulfate. The carbon source utilized was a mixture of acetate (3.48 g) and butyrate (0.54 g), equivalent to a COD

ratio of 3.5:1. The headspace (150 mL) was flushed with N_2 gas prior to the addition of the VFA in order to promote an anaerobic atmosphere. At the end of the incubation period samples were taken for sulfate and sulfide (HS^-) analysis. An estimate of the cell count was conducted by optical density measurement − extinction coefficient 2×10^9 cell $mL^{-1}cm^{-1}$ at λ 540 nm − based on McFarland scale.

2.4 Fed batch experiments with the inoculum for sulfate reduction evaluation

Prior to the inoculation of the MFC with the sulfidogenic sludge, sulfate reduction was evaluated at 30°C in a single experiment using a glass flask KMAX KIMBLE® (500 mL of working volume and 150 mL of headspace), the experiment was conducted under fed batch mode since the operation of the MFC was proposed to be in fed batch mode. A set of three fed batch experiments were conducted in order to assess the sulfate reduction in the inoculum. The experiment was conducted by duplicate in two glass flasks. Each one of the two glass flasks was inoculated with a mixture (sludge-sediment) at a concentration of 544 g VSS L^{-1} (0.022 g VSS g -1 $_{\text{sludge-sediment mixture}}$).

The number of incubation days for each batch was: 15 (batch 1), 14 (batch 2) and 12 (batch 3). Acetate and butyrate at a COD proportion of 3.5:1 were used as carbon source and the initial COD and sulfate concentrations were 4 and 6 g L -1, respectively. The initial pH for each batch was in a range of $3.5 - 4.5$. The COD/SO_4^{-2} ratio resulted as follows: 1, 0.67 and 1.5 for batch 1, 2 and 3, respectively. There was no addition of a buffer solution to the system and analysis for SO_4^{-2}, COD, HS^-, and pH were conducted to 7 different samples taken from each batch experiment. Volatile suspended solids (VSS) was analyzed on day 1 (0.026 g VSS g -1 $_{\text{sludge-sediment mixture}}$) prior to the beginning of batch 1 and on the last day of batch 3 (0.123 g VSS g -1 $_{\text{sludge-sediment mixture}}$).

2.5 Acclimation to acetate as the only electron donor in the microbial fuel cell (MFC)

After the conclusion of the sulfate reduction experiments in fed batch mode, a set of tests in a microbial fuel cell were conducted. The system that was utilized is shown in Fig. 1. The system consisted of two 500 mL chambers, an anodic chamber (anaerobic) and a cathodic chamber (aerobic).
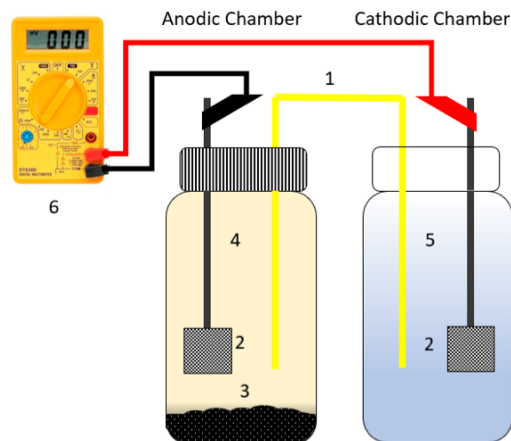

Fig. 1. Microbial fuel cell scheme. 1) salt bridge, 2) graphite cloth (electrode), 3) sulfidogenic sludge/sediment, 4) sulfate reducing medium, 5) water and 6) multimeter.

The anodic chamber was sealed and the cathodic chamber was open to the atmosphere. The area of the graphite electrodes (graphite cloth) was of 8 cm^2 and it was activated by immersion in HCl 1 M for 24 h, followed by washing with distilled water and a final immersion in a NaOH 1 M for another 24 h, finally the pH was adjusted to 7. The distance between both electrodes was of 6 cm. The chambers were connected with a salt bridge (NaCl 1 M) in agar. The anodic chamber contained the sulfate reducing inoculum (558 mg VSS L^{-1}) obtained from the acclimation mentioned in the former section, sulfate as the alternate electron acceptor (added from a stock solution at a concentration of 6000 mg L^{-1}), a mixture of VFA acetate:butyrate (added from a stock solution at a COD concentration of 4000 mg L^{-1}) and culture medium, to complete a final working volume of 500 mL. A buffer solution was not included. The Table 1 presents the VFA proportions used and periods of time for the gradual acclimation of the sludge from the acetate:butyrate mixture to only acetate. Each period was operated in fed batch mode. After each period the cell was washed with fresh medium to remove the remaining VFA, sulfate and sulfide before starting with the next VFA mixture. To wash the MFC, the inoculum and the graphite cloth were removed by draining them from the medium, after this, the MFC was washed with fresh medium without sulfate and without acetate. Once the MFC was washed, the inoculum and the graphite cloth were placed again in the cell. In order to avoid the loss of biomass in both, the inoculum and the graphite cloth, these were not washed.

Table 1. Acetate-Butyrate COD ratio in the MFC in the different periods of experimentation.

Period of experimentation	I	II	III	IV	V	VI
Days in each batch	0-12	13-25	26-38	39-51	52-64	65-77
Acetate-Butyrate COD ratio	3.5:1	3.7:0.8	3.9:0.6	4.1:0.4	4.3:0.2	4.5:0
Initial pH	4.75	4.65	5.85	5.7	5.95	5.05
Temperature (°C)	30	30	30	30	30	30

MFC abiotic, consisted of culture medium and acetate.

MFC acetate, consisted of acetate, culture medium and sludge without sulfate. This MFC was operated after day 77 when the sludge was acclimated to acetate as the single source of carbon.

MFC abiotic and MFC acetate were operated for 14 days.

In all cases: Sulfate (SO_4^{-2}) initial concentration in the MFC: 6000 mg L^{-1}, COD initial concentration in the MFC: 4000 mg L^{-1}

To assess the power (mV) of the MFC in absence of sulfate, a MFC was prepared with sludge, culture medium and acetate without sulfate (MFC acetate). An abiotic MFC (MFC abiotic) was also prepared with acetate and culture medium without sludge. The conditions of pH, temperature and concentration of sulfate and COD for the MFC are described in Table 1. The experiment was set in duplicate. For each period mentioned in Table 1, analysis for COD, sulfate, sulfide, pH and voltage were conducted. For the MFC abiotic and MFC acetate the only determination made was power (mV).

2.6 Polarization curve and power density curves

The efficiency of the MFC was evaluated by means of the construction of a polarization curve that presents the voltage as a function of the current density, and a power density curve, that presents the power as a function of current. The polarization and power density curves were conducted in the system with the last combination of electron donor and sulfate (period VI see Table 1, with acetate as the only electron donor and sulfate). This reaction corresponds to Eq. (1). At this time, the circuit was closed at the point in which the voltage obtained was the highest (day 12) and the curve was carried out with the following external resistances: 820, 2700, 4700, 5600 and 15000 Ω. Current and power densities were calculated with Eq. (2 to 5) and the Coulombic Efficiency (CE_{an}) was calculated according to Eq. (6).

$$CH_3COO^- + SO_4^{2-} + \frac{1}{2}H^+ \longrightarrow 2HCO_3^- + \frac{1}{2}HS^- + \frac{1}{2}H_2S \tag{1}$$
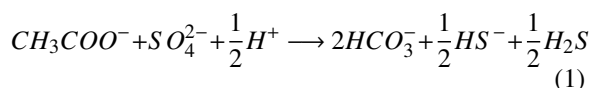
$$I = \frac{E}{R_{ext}} \tag{2}$$

$$Current\ density = \frac{I}{A_{an}} \tag{3}$$

$$Power(P) = I * E \tag{4}$$

$$Power\ density = \frac{P}{A_{an}} \tag{5}$$

$$CE_{an} = \frac{M \int_0^{tf} I dt}{Fb v_{an} \Delta COD} \tag{6}$$

where E is the voltage, R is the resistance, I is the current and A_{an} is the anode area, CE_{an} is the coulombic efficiency, M is the molecular weight of oxygen, F is the Faraday constant, b is the number of electrons exchanged, v_{an} is the volume of the anodic chamber and ΔCOD is the concentration of the COD removed.

2.7 Analytical methods

Sulfate, VSS and pH were analyzed according to standard methods (APHA, 2000). Sulfide (HS^-) was analyzed by the colorimetric method reported by Trüper and Schlegel (1964). The COD was determined by a commercial kit (HACH HR$^+$ range 0-15000 mg L^{-1}, USA) and a spectrophotometer (HACH, Germany) DR 2700. Voltage was measured with a commercial multimeter (STEREN, USA) MUL605. The sulfate removal rate (SRR, mg SO$_4^{-2}$ g^{-1} VSS L^{-1} d^{-1}) and sulfate reducing activity (SRA, mg COD-H$_2$S gVSS^{-1} d^{-1}) were evaluated in terms of initial sulfate concentration and COD/ SO$_4^{-2}$ ratio. SRR and SRA were calculated with equations (7) and (8), respectively.

$$SRR = \frac{(SO_4^{-2})_R}{VSS \cdot V} \tag{7}$$

$$SRA = \frac{(H_2S)_R}{VSS \cdot V} \qquad (8)$$

where $(SO_4^{-2})_R$ is the sulfate (SO_4^{-2}) reduction rate observed (mg SO_4^{-2} L^{-1} d^{-1}). VSS is the volatile suspended solids concentration (g VSS L^{-1}). $(H_2S)_R$ is the total sulfide production rate expressed in COD units (mg COD-H_2S L^{-1} d^{-1}) and V is the working volume of the MFC (L).

3 Results and discussion

3.1 Acclimation of the inoculum to sulfate reducing conditions

The mixture of sulfidogenic sludge and sediments (see materials and methods) was acclimated for 15 days to relatively high sulfate concentrations (7.5 g L^{-1}) and a COD concentration of 5 g L^{-1} (acetate and butyrate 3.5:1 at COD proportion), this corresponds to a COD/SO_4^{-2} ratio of 0.67. This acclimation period was maintained for a short time to simulate a scenario in which sudden changes in the concentrations of sulfate and carbon sources may occur and a diverse microbial community is present. During the acclimation period, 60% of sulfate was reduced, generating 75.5 mg L^{-1} of sulfide (Fig. 2a and b). On day 1, a 10% of sulfate reduction occurred and then it increased to 55% by day 9, after, a slight change in the percentage of sulfate reduction was observed until day 15 (60%, Fig. 2a),

which indicates that the acclimation of the mixture formed by sludge and sediment was accomplished. The acclimation time may vary among microbial communities, for example, Al Zuhair *et al*. (2008) mentioned that acclimation may be possible after 8 h, whereas Gacitúa *et al*. (2018) mentioned that some pure strain of SRB using lactate as electron donor (*Desulfovibrio paquessi* and *Desulfobacter halotolerans*) may reach 50% of sulfate reduction in 15 days against a SRB consortium that only requires 7 days. Bernardez *et al*. (2012) reported that a robust bed of sludge may require approximately 7 days to be formed.

In the present work, although sulfate reduction reached 60% on day 15, sulfate to sulfide conversion was low (75.5 mg L^{-1}), (Fig. 2b). The low conversion may be attributed to the low content of sulfate reducing microorganisms in the inoculum which at the beginning of the experiment consisted of a mixture of sludge and sediments hence the acclimation to sulfur reducing conditions was at an early stage. It is suggested that at this stage intermediates of the conversion of sulfate to sulfide were present (i.e., sulfite or thiosulfate) due to a low presence of SRB that could complete the reduction. It has been observed that sulfate to sulfide conversion increases in this type of sludge when the SRB are abundant and therefore, the *dsrA* gene (sulfite reductase subunit) is expressed (Santana- Santos *et al*., 2019). Another reason for a low sulfate conversion to sulfide could be the adsorption to the sedimentary portion of the sludge-sediment mixture.
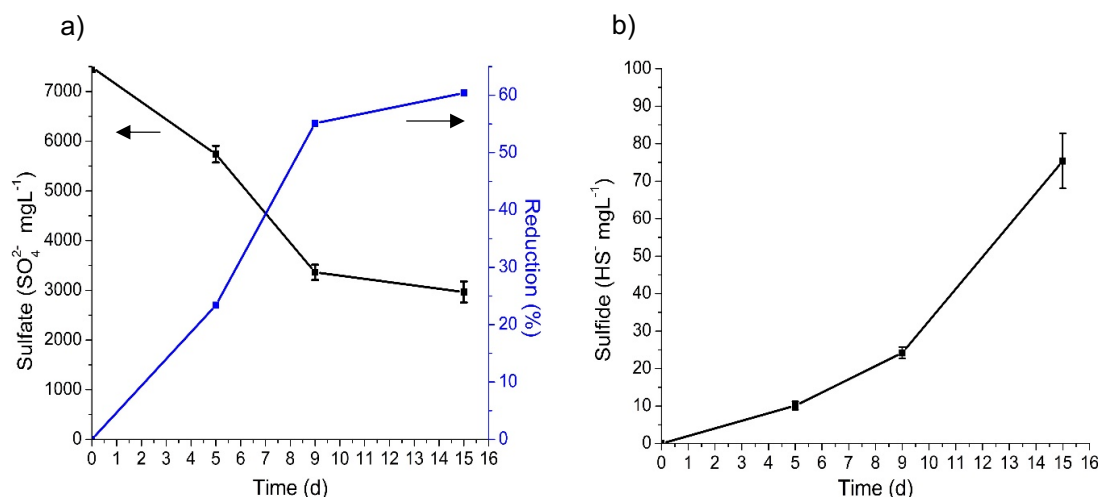
a)

b)

Fig. 2. Sulfate reduction during the acclimation period of the inoculum to sulfate reducing conditions. a) Sulfate (SO_4^{2-}) concentration (mg L^{-1}) over time, line in black, percentage of sulfate reduction (%), line in blue, and b) Sulfide (HS^-) concentration (mg L^{-1}) over time.

In addition, although at a lower extent, sulfate was assimilated to form biomass, as indicated by the cell density, which was of 4.2×10^8 cell mL^{-1} on day 1 and reached 7×10^8 cell mL^{-1} 16 days later. This is in agreement with the reported previously by other authors, i.e., Gacitúa *et al.* (2018), who reported that a pure strain of SRB may reach 50% of sulfate reduction in 15 days and a consortium may require 7 days. According to Bernardez *et al.* (2012) the stability in a sulfate reducing sludge may take approximately 7 days, as it was reported in their work in which a formation of biofilm was observed after 7 days using lactate as electron donor. At this point in the present work, the sludge was further cultivated under sulfate reducing conditions in batch experiments to observe the increase in sulfate reducing capability.

3.2 Sulfate reduction with the sludge as inoculum in fed batch experiments

The results obtained on sulfate and sulfide concentrations, COD and pH from the three batch experiments are presented in Fig. 3. The first batch (COD/SO$_4^{-2}$ = 1, days 1 − 15) presented a 74% of sulfate removal (equivalent to 36.43% of SO$_4^{-2}$ to sulfide conversion at pH 7.8) on day 15, with a SRR of 970 ± 7.7 mg SO$_4^{-2}$ g VSS^{-1} L^{-1} d^{-1}.
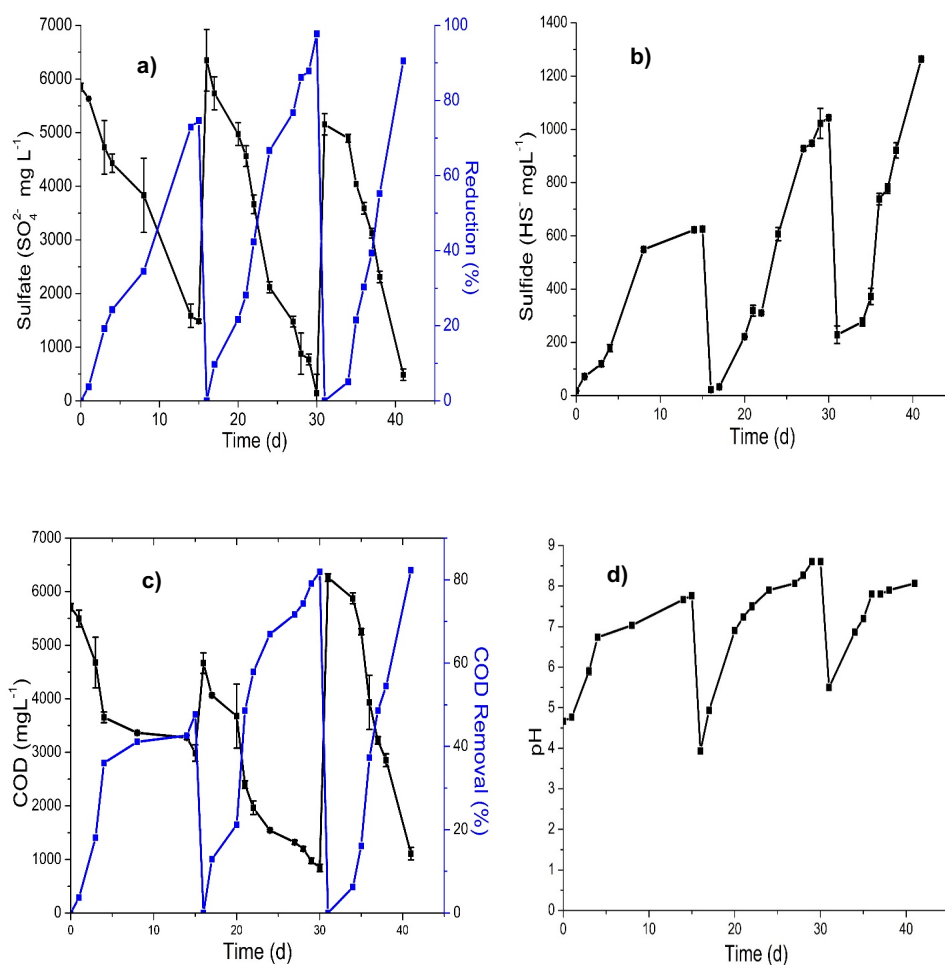

Fig. 3. Sulfate reduction in batch experiments with the acclimated inoculum. Sulfate (SO$_4^{-2}$), sulfide (HS$^-$), COD concentration over time (mg L^{-1}), and pH in the batch experiments. a) Sulfate (mg L^{-1}), b) Sulfide (mg L^{-1}), c) COD (mg L^{-1}), and d) pH.

The second batch (COD/SO$_4^{-2}$ = 0.67, days 16 − 29) presented a 98% of sulfate removal (equivalent to 60.1% of SO$_4^{-2}$ to sulfide conversion at pH 8.6) and a SRR of 1478 ± 50 mg SO$_4^{-2}$ g VSS^{-1} L^{-1} d^{-1}), and the third batch presented a 90% of sulfate removal (equivalent to 67.2% of SO$_4^{-2}$ to sulfide conversion at pH 8.1) on day 10 of the batch (COD/SO$_4^{-2}$ = 1.5, days 30 − 41) at a SRR of 1556 ± 21 mg SO$_4^{-2}$ g VSS^{-1} L^{-1} d^{-1}. The SRRs obtained in the second and third batches suggest steadiness in the sulfate reduction reaction.

Although the VFA and sulfate concentrations added to the system were selected to maintain COD/SO$_4^{-2}$ ratios of 1, 0.67 and 1.5, there were fluctuations of this ratio due to residual sulfate and/or COD in the system between each batch. The conversion of sulfate to sulfide (HS$^-$) was not influenced by the variation in the COD/SO$_4^{-2}$ ratio since the concentration of sulfide increased during the second (1043 mg L^{-1}) and third batches (1364 mg L^{-1}, Fig. 3b), which in both cases was higher than 60% conversion of sulfate to sulfide according to Eq. (1) and the pH equilibrium diagram for H$_2$S. Taking into account that acetate is the major component of the VFA mixture, this may indicate that the acclimation of the sludge was effective since in the first batch the sulfide concentration obtained initiated at 17.5 and reached 625.3 mg L^{-1} on the last day of the first batch (day 15). The COD removal obtained was of ~48% for the first batch on day 15, and 82% for the second (day 30) and third batch (day 40) (Fig. 3c). Analysis conducted during the first and last day of each batch showed that the fluctuations of COD concentration due to remaining COD between each batch caused that the concentrations of COD were slightly different that 4000 mg L^{-1}, which was the concentration fixed to perform the batches. Thus, it was observed that the highest COD removal occurred in batch 2 and 3 that started with COD

initial concentrations of ~4600 and 6200 mg L^{-1}, respectively (Fig. 3c). This was consistent with the percentage of sulfate removal (>90%) obtained in these batches.

The COD removal or consumption of the carbon source is affected by the pH during the culturing of sulfate reducing bacteria since the pH influences the metabolic processes such as homeostasis, transport of compounds in the cells and the dissociation of the electron donor (Koschorreck, 2008; Sanchez-Andrea *et al.* 2014; Janyasuthiwong *et al.*, 2015).

In the present work, the initial pH was of 4.7, 4 and 5.6 for batches 1, 2 and 3, respectively and after three days, the pH increased to 5.9, 6.9 and 6.9, respectively along with percentages of sulfate reduction of 19.2, 22 and 22.6%, respectively. This is in agreement with the sulfate reduction process in which the oxidation of organic matter yields HCO$_3^-$ and H$_2$S (H$_2$S aqueous and HS$^-$ are the sulfide species more abundant at pH < 7 (See Eq.1 in Materials and Methods section). Sulfate reducing bacteria (SRB) thrive in a pH range of 5 − 9 and the sulfate reducing activity diminishes at low pH (i. e. 2) (Jong and Parry, 2006; Neculita *et al.*, 2007). Regarding to the percentage of COD removal on day 3, it was of 18.1, 21 and 37% for each batch, respectively. At the end of each batch the final pH was of 7.6, 8.5 and 8.2, respectively (Fig. 3d). These results demonstrate that the sludge is capable of reducing sulfate with a source of carbon in which acetate is a major component.

3.3 Acclimation to acetate as the only electron donor in the Microbial fuel cell (MFC) in fed batch experiments

A gradual substitution of ~20% of butyrate by acetate was conducted in the MFC according to Table 1. The results obtained on sulfate and sulfide concentrations over time for each period are presented in Fig. 4.

Table 2. Sulfate reduction, sulfide production, COD removal and open circuit voltage (OCV) obtained from the system during the adaptation of the sludge to acetate as single electron donor.

Period and VFA mixture. (acetate/butyrate Ratio)	Operation days	Final HS$^-$ concentration (mg L^{-1})	Sulfate reduction (%)	SRR* (mg SO$_4$-^{2} gVSS^{-1} L^{-1} d^{-1})	(SO$_4^{2-}$) to H$_2$S Conversion (%)	COD removal (%)	SRA* (mg COD-H$_2$S gVSS^{-1} d^{-1})	Final OCV voltage (V)
I (3.5:1)	12	774.79 ± 4.24	72 ± 0.03	1755.28 ± 50	74.15 ± 0.39	66.35 ± 0.80	155.20 ± 30	0.668
II (3.7:0.8)	12	841.43 ± 5.09	67 ± 0.70	1490.98 ± 66	72.20 ± 0.43	61.43 ± 0.31	167.70 ± 21	0.716
III (3.9:0.6)	12	737.56 ± 98.4	62 ± 0.40	1509.37 ± 23	67.13 ± 4.13	65.18 ± 2.96	104.15 ± 15	0.716
IV (4.1:0.4)	12	860.35 ± 2.12	70 ± 1.29	1581.87 ± 77	75.37 ± 0.90	69.20 ± 0.32	118.98 ± 17	0.729
V (4.3:0.2)	12	1027.85 ± 0.42	63 ± 0.28	1422.44 ± 46	80.08 ± 0.42	72.56 ± 0.11	180.14 ± 23	0.738
VI (4.5:0)	12	1031.76 ± 0.84	70 ± 0.23	1692.31 ± 38	85.21 ± 0.34	81.35 ± 0.39	184.25 ± 24	0.788

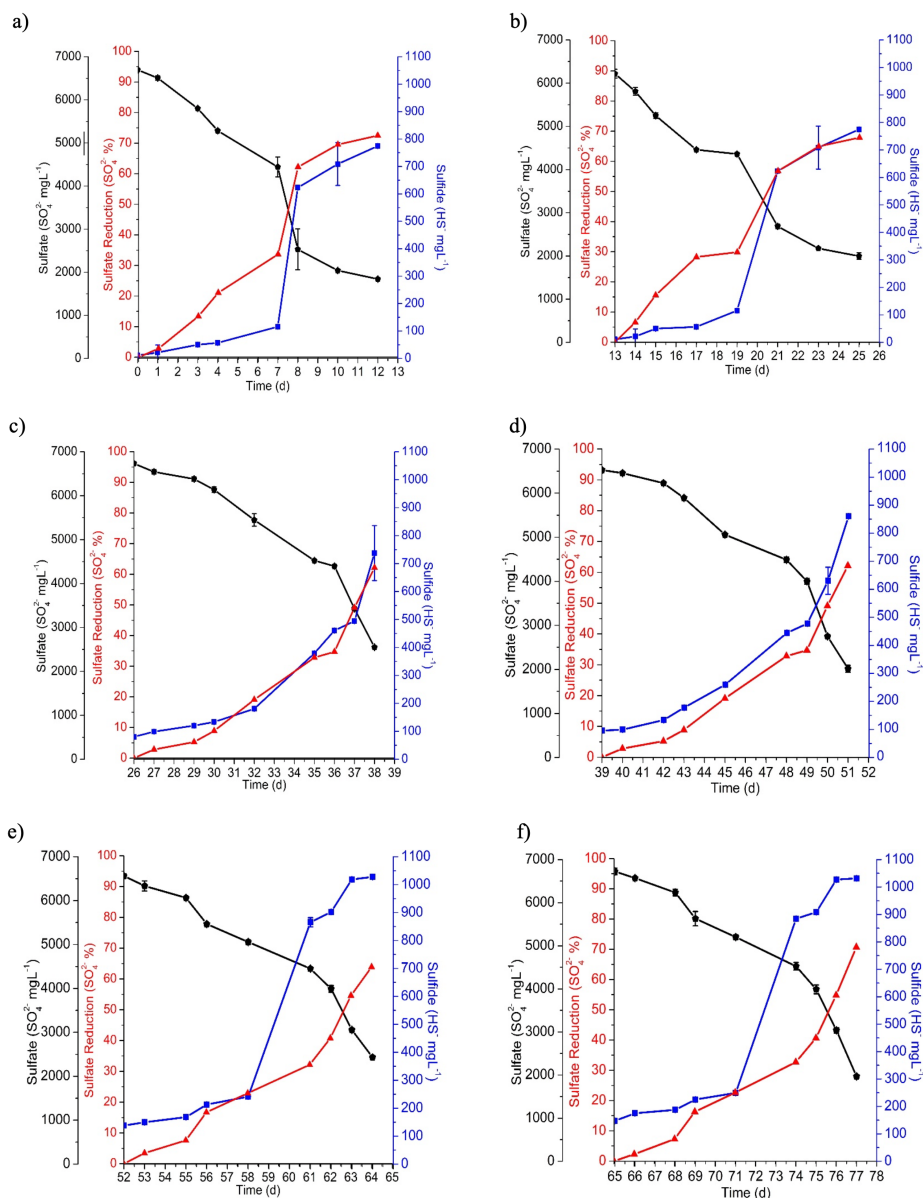*Sulfate removal rate (SRR); Sulfate reducing activity (SRA)

Fig. 4. Gradual acclimation of the sludge to acetate as the only source of carbon. Sulfate (SO_4^{-2}) and Sulfide (HS^-) over time for the different COD ratios of acetate and butyrate: a) ratio (I) 3.5 acetate:1 butyrate, b) ratio (II) 3.7 acetate :0.8 butyrate, c) ratio (III) 3.9 acetate:0.6 butyrate, d) ratio (IV) 4.1 acetate:0.4 butyrate, e) ratio (V) 4.3 acetate:0.2 butyrate and f) ratio (VI) 4.5acetate:0 butyrate. Line in black, sulfate (SO_4^{-2}) concentration mg L^{-1} , line in blue, sulfide (HS^-) concentration mg L^{-1} and line in red, percentage of sulfate reduction (%).

The results obtained on percentages of conversion of sulfate to sulfide and COD removal along with voltages obtained at open circuit (OCV) during all periods are presented in Table 2.

The results show that the percentage in sulfate reduction presented fluctuations through the periods in which the VFA changed in proportion but it remained almost the same at the two ratios 3.5:1 acetate butyrate (period I) and acetate only (4.5, period VI). The average sulfate reduction rate (SRR) obtained under the process that led to acetate as the single electron donor was of 1575 ± 127 mg SO_4^{-2} gVSS^{-1} L^{-1} d^{-1},

this value was almost the same as the obtained during the acclimation of the inoculum (1556 ± 21 mg SO_4^{-2} gVSS^{-1} L^{-1} d^{-1}), however, the conversion of sulfate to sulfide was higher when acetate was the only source of carbon, this is, a $85.21\pm0.34\%$ versus $74.15\pm0.39\%$ obtained at an acetate:butyrate ratio of 3.5:1 (Table 2). The percentage of sulfate reduction was about 70% in all periods whereas the percentage of sulfate to sulfide conversion was from ~70 to 85% (Table 2). These results suggest that over time the consortium was enriched in SRB that promote the conversion of sulfate to sulfide, particularly the conversion of the sulfite intermediate to sulfide, a conversion that is carried out by the *dsrA* gen expressed in SRB (Santana − Santos *et al.*, 2019).

The percentage of COD removal was $66.35 \pm0.80\%$ for a ratio of 3.5:1 acetate: butyrate (period I), whereas acetate as the only source of carbon presented $81.35 \pm 0.39\%$ of COD removal. This suggests that the consortium in the sludge was increasing in microorganisms capable of oxidizing acetate completely while reducing sulfate, this could also suggest an increase in sulfate-reducing bacteria that are considered "complete oxidizers".

A global overview of the concentrations reached for sulfide over the six periods (6 different VFA mixtures) is shown in Fig. 4. The sulfide concentration increased from 11.1 to 774.8 mg L^{-1} for the first period (Fig. 4a), which represented a sulfate reduction of 72%. As butyrate concentration was decreasing, sulfide concentration was increasing in the different periods, for example, initial concentrations ranged from ~80 (Fig. 4c) to 138 mg L^{-1} (Fig. 4e), whereas the final HS$^-$ concentrations ranged from

774.8 (Fig. 4a) to ~1032 mg L^{-1} (Fig. 4f) in 12 days. The percentage of sulfate reduction at the last period (4.5:0 acetate:butyrate) was 70%, not very different from the percentage reached at a 3:5:1 acetate:butyrate ratio (72%), however, the results obtained on conversion from sulfate to sulfide and COD removal suggest that sulfate reduction was driven for sulfate reducing bacteria that can oxidize acetate. A set of several batches of 12 and 15 days were conducted with acetate as the sole source of carbon some days after the acclimation to acetate. Those batches presented a steady sulfate reduction rate of 1104 mg SO$_4^{-2}$ g VSS^{-1} L^{-1} d^{-1} and an average of percentage on sulfate reduction up to 82% (data not shown). Although the rate of sulfate reduction decreased over time, it was observed that percentage of sulfate reduction and percentage of sulfate conversion to sulfide with acetate as electron donor were very close to the shown in Table 2. This implies that the sludge was successfully adapted to acetate and that it could be used for a steady system in which relatively high sulfate concentrations are used.

In regard to open circuit voltage (OCV), it ranged from 0.668 V with a $66.35 \pm 0.80\%$ of COD removal when the COD proportion was 3.5:1 acetate butyrate, to 0.788 V and $81.35 \pm 0.39\%$ of COD removal with acetate as the only source of carbon (Table 2). In the abiotic MFC, the maximum open circuit voltage reached in two weeks was of 0.2 V (200 mV) as it is shown in Fig 5a. The MFC in which acetate was supplemented in absence of sulfate (MFC acetate) presented an initial OCV of 0.25 V (250 mV) and reached in 10 days a maximum OCV of 0.45 V (450 mV), this OCV decreased over time as is shown in Fig. 5b.
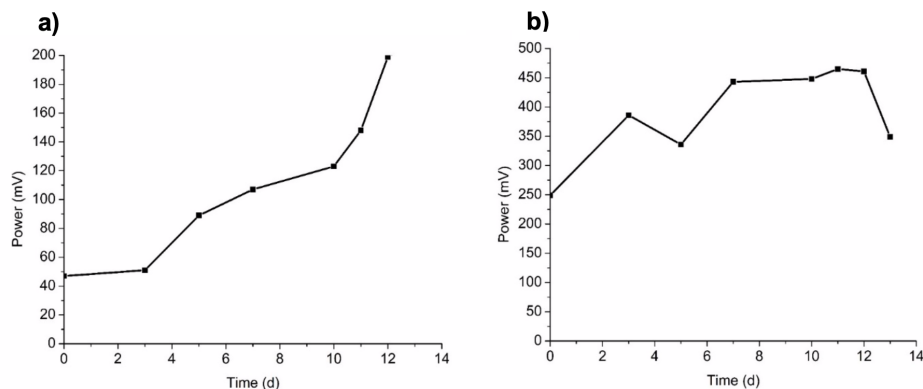

Fig. 5. Open circuit voltage (Power) in mV for a) MFC abiotic (a cell with medium and acetate, without sludge) and b) MFC acetate (a cell with sludge and acetate without sulfate).

The voltage in absence of sulfate was of 0.45 V compared to 0.788 V reached in the presence of sulfate. This indicates that sulfide is a catalyst in the generation of energy in the MFC. The comparison of the voltage observed in the MFC abiotic (from 50 to 200 mV) against the range of voltage obtained in the MFC acetate (250 to 450 mV) indicates that the sludge is also a catalyst in the generation of energy in the MFC. Taken these results altogether, it is suggested that both, the sludge and sulfide are involved in the electrogenic process. The SRB in the sludge carry out sulfate reduction and part of the sulfide generated serves as electron donor to the electrode. Sulfide oxidizes to sulfur in the anode abiotically and transfers 2 electrons. Sulfur produced could be oxidized to sulfate if the sulfur oxidizing microorganisms are present in the consortium (Lovley, 2006; Kumar *et al*., 2017a; Kumar *et al*., 2017b).

The sulfide concentrations reached in the present experiments were relatively high (~1000 mg L^{-1}), it has been considered that concentrations of sulfide 200 − 500 mg L^{-1} may be toxic to microorganisms (Sanchez − Andrea *et al*., 2014). If sulfide is not completely oxidized in the MFC it can always be used for example, for metals precipitation in an external unit. In the present case, large proportions of acetate were utilized as electron donor which implies that the sludge could be used in the MFC to eventually remove the COD in wastewater in which acetate is the residual product of complex organic matter. On the other hand, addition of acetate has been reported as strategy to improve the stability of MFC designed to treat domestic wastewater in concentrations up to 1 g L^{-1} (Stager *et al*., 2017), which suggests that inocula such as the sludge developed in the present work could be an alternative for that purpose.

The results obtained in the present work can be compared to the data presented in the report by Lee *et al*. (2014). These authors presented sulfate conversion to elemental sulfur (S0) in a range of ~48 to 78% in a MFC in which lactate was used as electron donor (~4.5 g L^{-1}) at an initial sulfate concentrations of ~250 mg L^{-1}. In that work, they obtained an OCV of 0.320 V during the first two weeks of acclimation of the activated sludge to the MFC conditions, whereas the OCV reached was of 0.730 V in experiments in which a stable biofilm was formed on the anode. In the present work an OCV of 0.788 V was obtained once the sludge was acclimated to acetate and sulfate. In addition, in this work higher sulfate and COD concentrations were tested in the experiments. Sulfide removal − through the development of a microbial community capable of oxidizing sulfide − was not the aim of this work, rather, the observations were focused on sulfate and COD removal with a high percentage in acetate.

3.4 Polarization curve with acetate as electron donor

In order to evaluate the performance of the MFC with acetate (100%) as electron donor, a polarization and power density curves were conducted. The results obtained from the polarization and power density curves conducted with acetate as the only electron donor are shown in Fig. 6. The resistances used to measure the power in the cell were: 820, 2700, 4700, 5600 and 15000 Ω. The MFC connected through a salt bridge and supplemented with acetate/sulfate in the anodic chamber presented a maximum OCV of 0.788 V and a maximum power density of (P_{max}) 0.266 mW (m^2)$^{-1}$. The Coulombic Efficiency (CE) was of 88.09%, this may indicate that a high percentage of electrons due to acetate may contribute to the generation of energy whereas the remaining electrons may have been directed to the generation of biomass or sulfate reduction based on the high conversion of sulfate to sulfide obtained. In the present case, the polarization curve was conducted during the last days of the batch in which the cell was fed with 100% acetate compared with a biofilm developed for longer periods of time in which OCV may reach 0.7 V and much higher power densities (Lee *et al*., 2012). On the other hand, it has also been observed that although biofilms may be developed on the electrode, as is the case of Lee *et al*. (2012), in which the power density was higher than the obtained in the present work, the COD removal was lower (24.3%) with 84% of sulfate reduction.
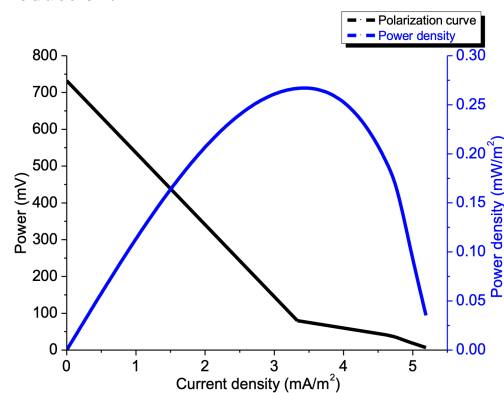

Fig. 6. Polarization and power curves with acetate and sulfate at the maximum OCV (788 mV).

Table 3. Electrochemical data and COD removal reported for work on MFC involving sulfate and different inocula.

Reference	Chou *et al.* (2013) MFC	Liang *et al.* (2013). MFC	Lee *et al.* (2014) MFC	Sangcharoen *et al.* (2015) MFC	Peng *et al.* (2017). MFC	Hu *et al.* (2019) MEC	This work (MFC)
MFC configuration	Cylindrical chambers ID: 4 cm. volume 100 mL	Single chamber	Double cylindrical Chambers anode/cathode	Single chamber (2 L)	Single vertical chamber (1 L)	Double chamber (30 mL)	Borosilicate bottles (500 mL)
Electrodes A: Anode C: Cathode	A and C: Carbon felt 1 cm x 1 cm x 1 cm	A and C: Carbon cloth 100 cm²	A and C: Carbon felt 9 cm²	A:30 cm² C: 25 cm². Carbon cloth	A and C: Carbon cloth 25 cm².	A: graphite fiber: 28274.33 mm³ C: graphite plaque 1250 mm³	A and C: Carbon cloth 16 cm².
Separator	CEM	No Membrane	CEM	PEM	PEM	CEM	Salt bridge (1 M NaCl)
Sulfate (SO_4^{-2}) Electron donors Microorganisms	200 mg/L lactate sludge	672 mg/L ethanol sludge	777 mg/L lactate sludge	1300 mg/L lactate SRB consortium	2100 mg/L acetate Anaerobic sludge and SRB consortium	2361 mg/L Sediments and SRB in the cathode	6000 mg/L Acetate SRB consortium
Sulfate reduction Sulfide (HS^-)	66 % 7 mg/L	82 % NR	77.9 % 172.3 mg/L	18 % 91.1 mg/L	71.2 % 18 mg/L	49 % 35.7 mg/L	70 % 1031.8 ± 0.84 mg/L
COD removal OCV (mV) Current density (mA/m²) Power density (mW/m²)	93.8% 600 300 5	~72% NR 500 (calculated) NR	NR 730 NR 62	57% NS NR NR	51.6% 413 43 51.3	NR NR ~2.28 x10⁵ § NR	81.34 ± 0.4 % 788 3.33 0.266

NR not reported; NS not specified; CEM: cation exchange membrane; PEM: proton exchange membrane. § surface area of the electrode was estimated from the data provided in the document.

This was attributed to the resistance reached in the biofilm once it had reached its maximum capability for transferring electrons (Lee *et al.*, 2012).

In general, the factors that influence the most the polarization curve are: the electrode surface area (Zhao *et al.*, 2009), the biofilm (Lee *et al.*, 2012; Peraza − Baeza *et al.*, 2016), the acetate concentration, in which case a higher acetate concentration favors the performance of the cell (Khater *et al.*, 2017; Stager *et al.* 2017), the accumulation of VFA that may cause acidification of the medium (Domínguez − Maldonado *et al.*, 2014), a combination of lower sulfate concentration than electron donor concentration (Lee *et al.*, 2014; Weng *et al.*, 2015) and the acetate/sulfate ratio (Sharma *et al.*, 2013). Based on the results obtained in our case, some design factors can be further studied to improve the system. For example, although it has been reported that a salt bridge indeed facilitates the transferring of protons from anode to cathode and that it may contribute to a COD removal improvement (Sevda and Sreekrishnan, 2012), it may be necessary to assay different shapes and distances between the chambers connected with the bridge. Although this work was developed according to the suggestions of authors that have reported improvements on COD removal and voltage in MFC utilizing salt bridge (Sevda and Sreekrishnan, 2012; Muralidharan *et al.*, 2011). Sevda and Sreekrishnan (2012) reported that a concentration of salt higher than 0.85 M improves the performance and conductivity of the system. An ideal concentration of salt in the salt bridge to remove COD without affecting the current obtained in the system is 1 M according to Muralidharan *et al.* (2011), whereas a higher concentration exerts a negative effect on the voltage of the cell. In the present work, 1 M NaCl was used in the salt bridge. Table 3 summarizes the performance of the MFC studied here in comparison to different reports published by other authors that have worked with MFC that include sulfate. It can be seen that in the present work a higher sulfate reduction is obtained coupled to a higher sulfide concentration produced and a higher COD removal with an OCV of 0.788 V. However, the power and current densities are lower due to design constraints as mentioned above. Further experiments that include larger surface area of the anode and a higher development of the biofilm are required. According to Peng *et al.* (2017), a more dense biofilm with a distance between electrodes of ∼6 cm or slightly shorter would favor sulfate reduction and electrons transfer.

Conclusions

The approach of this work was the cultivation of a sludge to reach efficient sulfate reduction for potential applications in sulfate rich wastewater treatment and generation of energy. It was observed that an increase of 22% in acetate as electron donor by substitution of butyrate (from 3.5:1 (acetate − butyrate) to 4.5 (acetate) as COD proportion) in the sulfidogenic sludge resulted in higher COD removal (from 66.35 ± 0.80 to 81.35%) and sulfate reduction

(from 66- to 77%) in comparison to the combination acetate-butyrate. A higher sulfate to sulfide conversion was also achieved with acetate ($85.21 \pm 0.34\%$ and SRA of 184.25 ± 24 mg COD-H_2S gVSS^{-1} d^{-1}) than with the combinations acetate-butyrate (~ 67 to 74%) although the sulfate removal rate does not increase when acetate is the main electron donor. The open circuit voltage (OCV) reached in the present work was of 0.788 V, this was higher with acetate as the only source of carbon than with the mixture acetate − butyrate. This OCV was higher than the obtained in other works conducted with sulfate (and lactate) in MFC. As for the power and current densities, these were lower compared to other reports. The results suggest that is feasible the utilization of the sludge in a MFC system with acetate and sulfate to carry out sulfate reduction and COD removal, the generation of energy may be improved by modifying some aspects of the system that would favor the oxidation of sulfur species on the anode, such as the area of the electrodes and the development of biofilm on the anode.

Acknowledgements

Abbreviations

UASB	Upflow anaerobic sludge blanket reactor
MFC	microbial fuel cell
COD	chemical oxygen demand
VFA	volatile fatty acids
OCV	open circuit voltage, V
SRR	Sulfate reducing rate, mg SO_4^{-2} gVSS^{-1} L^{-1} d^{-1}
SRA	Sulfate reducing activity, mg COD-H_2S gVSS^{-1} d^{-1}
VSS	Volatile suspended solids, mg L^{-1} or g L^{-1}
CE$_{an}$	Coulombic efficiency, %
SRB	Sulfate reducing bacteria

Greek symbols

Ω	ohms

References

Angelov, A., Bratkova, S. and Loukanov, A. (2013). Microbial fuel cell based on electroactive sulfate-reducing biofilm. *Energy Conversion and Management 67*, 283-286. https://doi.org/10.1016/j.enconman.2012.11.024

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

APHA. (2005). Standard Methods for examination of water and wastewater. *American Public Health Association*, USA.

Bernardez, L., Lima, L. R. P., Ramos, C. L. S. and Almeida, P. F. (2012). A kinetic analysis of microbial sulfate eeduction in an upflow packed-bed anaerobic bioreactor. *Mine Water and the Environment 31*, 62-68. https://doi.org/10.1007/s10230-012-0170-z

Chou, T. Y., Whiteley, C. G., Lee, D. J. and Liao, Q. (2013). Control of dual-chambered microbial fuel cell by anodic potential: Implications with sulfate reducing bacteria. *International Journal of Hydrogen Energy 38*, 15580-15589. https://doi.org/10.1016/j.ijhydene.2013.04.074

Do, M.H., Ngo, H.H., Guo, W.S., Liu, Y., Chang, S.W., Nguyen, D.D. (2018). Challenges in the application of microbial fuel cell to waste water treatment and energy production: a mini review. *Science of the Total Environment 639*, 910-920.

Domínguez-Maldonado, J. A., García-Rodríguez, O., Aguilar-Vega, M., Smit, M., Alzate-Gaviria, L. (2014). Disminución de la capacidad de intercambio catiónico en una pila de combustible microbiana y su relación con la densidad de potencia. *Revista Mexicana de Ingeniería Química 13*, 527-538. http://rmiq.org/iqfvp/Pdfs/Vol.13,No.2/IA3/RMIQTemplate.pdf

Feng, C., Hou, C.H., Chen, S., Yu, C.P. (2013). A microbial fuel cell driven capacitive deionization technology for removal of low level dissolved ions. *Chemosphere 91*, 623-628. https://doi.org/10.1016/j.chemosphere.2012.12.068

García-Solares, S.M., Ordaz, A., Monroy-Hermosillo. O., Jan-Roblero, J. and Guerrero-Barajas, C. (2014). High sulfate reduction efficiency in a UASB using an alternative source of sulfidogenic sludge derived from hydrothermal vents sediments. *Applied Biochemistry and Biotechnology 174*, 2919-2940. doi:10.1007/s12010-014-1237-z

Gacitúa, M. A., Muñoz, E. and González, B. (2018). Bioelectrochemical sulphate reduction on batch reactors: Effect of inoculum-type and applied potential on sulphate consumption and pH. *Bioelectrochemistry 119*, 26-32. https://doi.org/10.1016/j.bioelechem.2017.08.006

Guerrero-Barajas, C. and García-Peña, E. I. (2010). Evaluation of enrichments of sulfate reducing bacteria from pristine hydrothermal vents sediments as potential inoculum for reducing trichloroethylene. *World Journal of Microbiology and Biotechnology 26*, 21-32.

Hao, T.W., Xiang, P., Mackey, H.R., Chi, K., Lu, H., Chui, H.K., van Loosdrecht, M.C.M. and Chen, G.H. (2014). A review of biological sulfate conversions in wastewater treatment. *Water Research 65*, 1-21.

He, L., Du, P., Chen, Y., Lu, H., Cheng, X., Chang, B. and Wang, Z. (2017). Advances in microbial fuel cells for wastewater treatment. *Renewable and Sustainable Energy Reviews 71*, 388-403.

Hu, J., Zeng, C., Liu, G., Lu, Y., Zhang, R. and Luo, H. (2019). Enhanced sulfate reduction accompanied with electrically-conductive pili production in graphene oxide modified biocathodes. *Bioresource Technology 282*, 425-432. https://doi.org/10.1016/j.biortech.2019.03.023

Janyasuthiwong, S., Rene, E. R., Esposito, G. and Lens, P. N. L. (2015). Effect of pH on the performance of sulfate and thiosulfate-fed sulfate reducing inverse fluidized bed reactors. *Journal of Environmental Engineering*, 1-11. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001004

Kaksonen, A. H. and Puhakka, J. A. (2007). Sulfate reduction based bioprocesses for the treatment of acid mine drainage and the recovery of metals. *Engineering in Life Sciences, 7*, 541-564. https://doi.org/10.1002/elsc.200720216

Karra, U., Troop, E., Curtis, M., Scheible, K., Tenaglier, C., Patel, N., *et al.* (2013) Performance of plug flow microbial fuel cell (PF-MFC) and complete mixing microbial fuel cell (CM-MFC) for wastewater treatment and power generation. *International Journal of Hydrogen Energy 38*, 5383-5388. https://doi.org/10.1016/j.ijhydene.2013.02.092

Khater, D. Z., El-Khatib, K. M. and Hassan, H. M. (2017). Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation. *Journal of Genetic Engineering and Biotechnology 15*, 127-137. https://doi.org/10.1016/j.jgeb.2017.01.008

Koschorreck, M. (2008). Microbial sulphate reduction at a low pH. *FEMS Microbiology Ecology 64*, 329-342. https://doi.org/10.1111/j.1574-6941.2008.00482.x

Kumar, S.S., Malyan, S.K., Basu, S., Bishnoi, N.R. (2017a), Syntrophic associationn and performance of Clostridium, Desulfovibrio, Aeromonas and Tethathiobacter as anodic biocatalysts for bioelectricity generation in a dual chamber microbial fuel cell. *Environmental Science and Pollution Research 24*, 16019-16030.

Kumar, S.S, Basu, S., Bishnoi, N.R. (2017b). Effect of cathode environment on bioelectricity generation using a novel consortium in anode side of a microbial fuel cell. *Biochemical Engineering Journal 121*, 17-24. https://doi.org/10.1016/j.bej.2017.01.014

Kumar, S.S., Kumar, V., Malyan, S.K., Sharma, J., Mathimani, T., Maskarenj, M.S., Ghosh, P.C., Pugazhendhi, A. (2019). Microbial fuel cells (MFCs) for bioelectrochemical treatment of different wastewater streams. *Fuel 254*, 115526. Doi: doi.org/10.1016/j.fuel.2019.05.109

Lee, D. J., Lee, C. Y. and Chang, J. S. (2012). Treatment and electricity harvesting from sulfate/sulfide-containing wastewaters using microbial fuel cell with enriched sulfate-reducing mixed culture. *Journal of Hazardous*

Materials 243, 67-72. https://doi.org/10.1016/j.jhazmat.2012.09.071

Lee, D. J., Liu, X. and Weng, H. L. (2014). Sulfate and organic carbon removal by microbial fuel cell with sulfate-reducing bacteria and sulfide-oxidising bacteria anodic biofilm. *Bioresource Technology 156*, 14-19. https://doi.org/10.1016/j.biortech.2013.12.129

Liang, F., Xiao, Y., and Zhao, F. (2013). Effect of pH on sulfate removal from wastewater using a bioelectrochemical system. *Chemical Engineering Journal 218*, 147-153. https://doi.org/10.1016/j.cej.2012.12.021

Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S. and Rabaey, K. (2006). Microbial fuel cells: methodology and technology. *Environmental Science and Technology 40*, 5181-5192. https://doi.org/10.1021/es0605016

Lovley, D.R. (2006). Bug juice: Harvesting electricity with microorganisms. *Nature reviews/Microbiology 4*, 497-508. doi:10.1038/nrmicro1442

Mohd Yusoff, M.Z., Hu, A., Feng, C., Maeda, T., Shirai, Y., Hassan, M.A. *et al.* (2013). Influence of pretreated activated sludge for electricity generation in microbial fuel cell application. *Bioresource Technology 145*, 90-96. https://doi.org/10.1016/j.biortech.2013.03.003

Muralidharan, A., Babu, A., Nirmalraman, K. and Ramya, M. (2011). Impact of salt concentration on electricity production in microbial hydrogen-based salt bridge fuel cells. *Indian Journal of Fundamental and Applied Life Sciences 1*, 2231-6345.

Neculita, C.M., Zagury, G. J. and Bussière, B. (2007). Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria. *Journal of Environment Quality 36*, 1. https://doi.org/10.2134/jeq2006.0066

Peng, X., Tang, T., Zhu, X., Jia, G., Ding, Y., Chen, Y., Yang, Y. and Tang, W. (2017). Remediation of acid mine drainage using microbial fuel cell based on sludge anaerobic fermentation. *Environmental Technology (United Kingdom) 38*, 2400-2409. https://doi.org/10.1080/09593330.2016.1262462

Peraza-Baeza, I., Pérez-Hernandez, A., Blanco-Cocom, L., Domínguez-Maldonado, J., Alzate-Gaviria, L. (2016). A mathematical model of the oscillations of pH for the anodic biofilm formation in microbial fuel cell. *Revista Mexicana de Ingeniería Química 15*, 763-771. http://rmiq.org/iqfvp/Pdfs/Vol.15,3/Bio8/RMIQTemplate.pdf

Sánchez-Andrea, I., Sanz, J. L., Bijmans, M. F.M. and Stams, A. J. M. (2014). Sulfate reduction at low pH to remediate acid mine drainage. *Journal of Hazardous Materials 269*, 98-109. https://doi.org/10.1016/j.jhazmat.2013.12.032

Sangcharoen, A., Niyom, W. and Suwannasilp, B. B. (2015). A microbial fuel cell treating organic wastewater containing high sulfate under continuous operation: Performance and microbial community. *Process Biochemistry 50*, 1648-1655. https://doi.org/10.1016/j.procbio.2015.06.013

Santana - Santos, M.A., Ordaz, A., Jan-Roblero, J., Bastida- González, F., Zárate- Segura, P.B. and Guerrero - Barajas, C. (2019). Tolerance of a sulfidogenic sludge to trichloroethylene at microcosms level as a basis for a long-term operation of reactors designed for its biodegradation. *Journal of Environmental Science and Health Part A 54*, 461-471.

Stager, J., Zhang, X. and Logan, B.E. (2017). Addition of acetate improves stability of power generation using microbial fuel cells treating domestic waste water. *Bioelectrochemistry 118*, 154-160. http://dx.doi.org/10.1016/bioelechem.2017.08.002

Sevda, S. and Sreekrishnan, T. R. (2012). Effect of salt concentration and mediators in salt bridge microbial fuel cell for electricity generation from synthetic wastewater. *Journal of Environmental Science and Health, Part A 47*, 878-886. https://doi.org/10.1080/10934529.2012.665004

Sevda, S., Domínguez-Benetton, X., Vanbroekhovenk, K., De wever, H., Sreekrishnan, T.R., Pant, D. (2013). High strength waste water treatment accompanied by power generation using air cathode microbial fuel cell. *Applied Energy 105*, 194-206. https://doi.org/10.1016/j.apenergy.2012.12.037

Sharma, M., Jain, P., Varanasi, J. L., Lal, B., Rodríguez, J., Lema, J. M. and Sharma, P. M. (2013). Enhanced performance of sulfate reducing bacteria based biocathode using stainless steel mesh on activated carbon fabric electrode. *Bioresource Technology 150*, 172-180. https://doi.org/10.1016/j.biortech.2013.09.069

Sivasankar, V., Mylsamy, P. and Omine, K. (2018). *Microbial Fuel Cell Technology for Bioelectricity*. Springer, Switzerland.

Trüper, H.G. and Schlegel, H.G. (1964). Sulphur metabolism in Thiorhodaceae I. Quantitative measurements on growing cells of *Chromatium okenii. Antonie Van Leeuwenhoek 30*, 225-238.

Van den Brand, T. P. H., Roest, K., Chen, G. H., Brdjanovic, D. and van Loosdrecht, M. C. M. (2015). Potential for beneficial application of sulfate reducing bacteria in sulfate containing domestic wastewater treatment. *World Journal of Microbiology and Biotechnology 31*, 1675-1681. https://doi.org/10.1007/s11274-015-1935-x

Weng, H. L. and Lee, D. J. (2015). Performance of sulfate reducing bacteria-microbial fuel cells: Reproducibility. *Journal of the Taiwan Institute of Chemical Engineers 56*, 148-153. https://doi.org/10.1016/j.jtice.2015.04.028

Zhao, F., Rahunen, N., Varcoe, J.R., Chandra, A., Avignone-Rossa, C., Thumser, A.E., *et al*. (2008). Activated carbon cloth as anode for sulfate removal in a microbial fuel cell. *Environmental Science Technology 42*, 4971-4876. https://doi.org/10.1021/es8003766

Zhao, F., Slade, R. C. T. and Varcoe, J. R. (2009). Techniques for the study and development of microbial fuel cells: An electrochemical perspective. *Chemical Society Reviews 38*, 1926-1939. https://doi.org/10.1039/b819866g