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Anaerobic treatment of sulfate-rich wastewater in an anaerobic sequential batch reactor (AnSBR) using butanol as the carbon source

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A B S T R A C T

Biological sulfate reduction was studied in a laboratory-scale anaerobic sequential batch reactor (14 L) containing mineral coal for biomass attachment. The reactor was fed industrial wastewater with increasingly high sulfate concentrations to establish its application limits. Special attention was paid to the use of butanol in the sulfate reduction that originated from melamine resin production. This product was used as the main organic amendment to support the biological process. The reactor was operated for 65 cycles (48 h each) at sulfate loading rates ranging from 2.2 to 23.8 g SO_4^{2-}/cycle, which corresponds to sulfate concentrations of 0.25, 0.5, 1.0, 2.0 and 3.0 g SO_4^{2-} L^{-1}. The sulfate removal efficiency reached 99% at concentrations of 0.25, 0.5 and 1.0 g SO_4^{2-} L^{-1}. At higher sulfate concentrations (2.0 and 3.0 g SO_4^{2-} L^{-1}), the sulfate conversion remained in the range of 71–95%. The results demonstrate the potential applicability of butanol as the carbon source for the biological treatment of sulfate in an anaerobic batch reactor.

1. Introduction

Sulfuric acid is used in several industrial processes, and as a result, sulfate is present in the wastewater of these processes (Muyzer and Stams, 2008). Wastewater containing sulfate is normally treated using physicochemical and biological methods (Greben et al., 2000; Sarti et al., 2010). Even though physicochemical methods are effective, their limitations restrict their usage. These limitations include the need for separation and appropriate disposal of the solid phase and relatively high costs and energy consumption (Silva et al., 2002; Bayrakdar et al., 2009). On the other hand, the success of high-rate anaerobic technology has encouraged researchers to extend its application to the treatment of complex wastewaters (Sarti et al., 2009).

The presence of high sulfate concentrations in specific wastewater restricts the application of anaerobic treatment technology because toxic, corrosive and odorous hydrogen sulfide (H_2S) is produced (Shayegan et al., 2005; Muyzer and Stams, 2008). H_2S is generated from the proliferation of sulfate-reducing bacteria (SRB) in anaerobic bioreactors, where they compete with methane-producing archaea (MPA) and homoacetogenic bacteria (AB) for common substrates (Mohan et al., 2005). The SRB have the ability to couple the oxidation of organic matter (electron donor) to the reduction of sulfate (electron acceptor), and they depend on hydrolytic and fermentative bacteria that degrade complex organic matter (Muyzer and Stams, 2008; Celis-Garcia et al., 2009; Damianovic and Foresti, 2009).

The efficiency of sulfidogenesis, however, can be strongly influenced by the characteristics and number of electron donors (Weijma et al., 2003; Zhao et al., 2010), especially if the latter is expressed as the chemical oxygen demand (COD)/sulfate ratio. For example, the COD/sulfate ratio has been studied in an attempt to establish the electron flux for sulfate removal from wastewater containing low concentrations of organic matter and for the combined removal of organic matter and sulfate (Lens et al., 1998; Velasco et al., 2008; Damianovic and Foresti, 2009).

Sulfate-reducing processes open up new possibilities for the anaerobic treatment of sulfate-rich wastewaters because SRB can metabolize several substrates of environmental interest, including organic toxicants (aromatics, alkanes, and chlorinated compounds), long chain and branched fatty acids, higher alcohols, and lactate (Celis-Garcia et al., 2007; Sarti et al., 2010). The range of electron donors (higher alcohols) that are known to be metabolized by sulfate reducers are propanol-1 and -2, butanol-1 and -2, isobutanol, pentanol, ethylene glycol, 1-2 propanediol, 1-3 propanediol and glycerol (Hansen, 1994). However, the use of butanol as the main organic source for sulfate reduction has not been described in the literature for wastewater treatment (industrial) in anaerobic reactors. The commonly used electron donors are hydrogen,

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methanol, ethanol, acetate, lactate, propionate, butyrate, sugar and molasses (Liamleam and Annachhatre, 2007).

A conventional anaerobic sequential batch reactor is operated with intermittent cycles of four stages: the feeding or loading process of liquid influent, anaerobic biological reactions, biomass sedimentation and effluent discharge (Sarti et al., 2007; Damasceno et al., 2007). Additionally, the use of inert supports to immobilize cells in sequencing batch reactors appears to be a promising method to improve the retention of solids, suppress the settling step and thus reduce the total cycle time (Miqueleto et al., 2005). According to Sarti et al. (2009), mineral coal can be considered an effective inert support for biomass attachment for methanogenic archaea and SRB. In this research, the application of biological treatments to industrial effluent containing high sulfate concentrations was carried out in a batch reactor with ethanol as the organic matter for sulfate reduction.

The study presented in this article investigates the optimization of COD/sulfate ratio for biological sulfate reduction in a bench-scale anaerobic sequential batch reactor with the use of mineral coal (inert support) and an electron donor obtained from the manufacture of melamine resin (butanol). Changes in the experimental conditions, such as in the influent sulfate concentration and COD/sulfate ratio, may lead to a better understanding of batch reactor operation for the treatment of sulfate-rich wastewater from an industrial process (sulfonation process).

2. Materials and methods

2.1. Wastewater characteristics

The wastewater from a chemical industry in which the sulfonation of vegetable oils (rice, soy and corn) is one of the most important wastewater-producing process. The wastewater originates from washing the products of the sulfonation reaction that occurs in the presence of sulfuric acid (H_2SO_4) and liquid ammonia (25%) in a batch reactor operated under a controlled temperature. The composition of the washing wastewater containing high levels of sulfate is presented in Table 1. In the same industry, the production of melamine resin in a batch reactor generates wastewater with a high concentration of butanol (99%). The butanol (~155 g COD L^{-1}) was used as the organic source for the sulfate reduction.

2.2. Anaerobic sequential batch reactor configuration and operation

The anaerobic batch reactor (AnSBR) was fabricated in the laboratory from acrylic material with the reactor had a total working volume of 14 L and had an internal diameter of 0.15 m with an L (length)/D (diameter) ratio of 6.0. The AnSBR was operated in the biofilm configuration in the upflow mode. Mineral coal (10–20 mm in diameter) was used as the inert material to support biofilm growth, and the fixed bed had a void ratio of 0.54 and a bed height of 0.70 m. The outlet biogas tube from the headspace (2 L) was immersed in a hydraulic seal (0.25 L) containing an alkaline solution (NaOH) for H_2S removal. A schematic representation of the batch reactor is given in Fig. 1.

Table 1
Characteristics of the industrial wastewater (20 samples).

Variables	Minimum	Maximum	Mean
pH	2.30	3.20	–
COD_{Total} (g L^{-1})	9.20	15.40	12.70 ± 4.1
$COD_{Filtered}$ (g L^{-1})	9.80	10.90	10.60 ± 1.3
SO_4^{2-} (g L^{-1})	180	284	201 ± 35

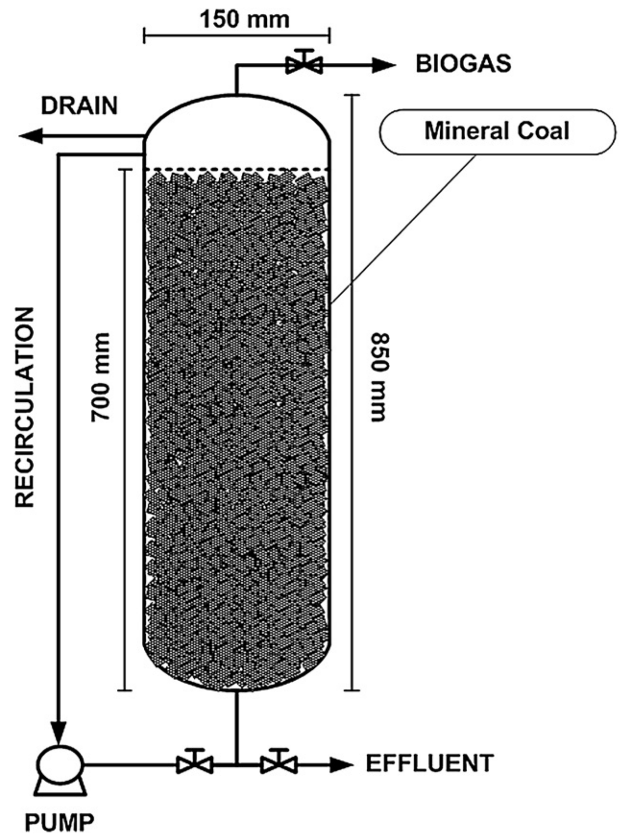

Fig. 1. Schematic representation of the anaerobic batch reactor containing biomass immobilized in mineral coal.

The total operational cycle was 48 h and included the following steps: feeding (1 h), reaction with continuous recirculation (46 h) and drainage (1 h). Agitation was maintained by ascending liquid recirculation (60 L h^{-1}) using a peristaltic pump (EMEC/CMS/02-06) and an ascensional velocity of 6.0 m h^{-1}. The reactor was operated in the sequencing batch mode at a constant temperature of 25 ± 1 °C, and the sequence phase periods (feed, reaction and drainage) during the batch reactor operation were controlled manually.

At the beginning of each cycle, a predefined feed volume (7.5 L) was pumped into the system, and the reactor volume was recirculated during the reaction phase. At the end of the reaction phase, the treated wastewater was withdrawn from the reactor. Table 2 summarizes the operational parameters (influent) applied to the anaerobic batch reactor in different experimental conditions (five periods). To achieve the sulfate and COD concentrations, dilution of the industrial effluent containing sulfate with tap water and the addition of a carbon source (wastewater from melamine resin production/butanol) were necessary to achieve sulfate loading rate (SLR) of 2.2, 4.3, 7.9, 15.7 and 23.8 g/cycle or sulfate concentrations of 0.25, 0.5, 1.0, 2.0 and 3.0 g SO_4^{2-} L^{-1}. The added volume of butanol (as COD) was varied according to the sulfate removal efficiencies obtained for the different COD/sulfate ratios (Table 2) to maximize the simultaneous sulfate reduction and butanol utilization in terms of COD.

2.3. Inoculation

Initially, the AnSBR was inoculated with 3.5 L of anaerobic sludge (37.2 g TS L^{-1} and 27.1 g TVS L^{-1}) taken from a full-scale UASB treating effluent from a poultry slaughterhouse. Tap water was pumped into the unit to reach the necessary reactor volume for

Table 2
Summary of the average operational parameters (influent) applied to the AnSBR. SLR = sulfate loading rate; OLR = organic loading rate.

Variables	Period I	Period II	Period III	Period IV	Period V
Cycle numbers	16	10	22	11	6
SO_4^{2-} (mg L^{-1})	289 ± 26	572 ± 70	1048 ± 72	2092 ± 75	3179 ± 95
SRL (g SO_4^{2-}/cycle)	2.2	4.3	7.9	15.7	23.8
COD[a]/sulfate	2.60 ± 0.54	3.09 ± 0.22	3.67 ± 0.45	3.65 ± 0.30	3.67 ± 0.19
COD_{Total} (mg L^{-1})	762 ± 205	1757 ± 132	3839 ± 442	7614 ± 538	11652 ± 763
OLR (g COD_{Total}/cycle)	5.7	13.2	28.8	57.1	87.4
BA (mgCaCO_3^{-2} L^{-1})	14.2 ± 11	18.8 ± 4.0	15.7 ± 8.0	15.2 ± 4.4	17.8 ± 5.4
VFA (mg Hac L^{-1})	20.7 ± 10.8	17.7 ± 3.9	29.8 ± 9.8	26.5 ± 4.1	22.7 ± 3.6
pH	6.2–7.4	6.5–7.4	6.6–7.5	5.9–7.0	5.9–6.5

[a] COD_{Total}.

treatment (4.0 L). Liquid recirculation continued in the reactor for 3 days. The total concentration of solids in the start-up period of the batch reactor was 17.4 g TS L^{-1} and 12.6 g TVS L^{-1} (TVS/TS = 0.72), considering the treated liquid volume (7.5 L).

2.4. Anaerobic sequential batch reactor monitoring

Monitoring (65 cycles) was carried out through physical–chemical analysis of the influent and effluent samples. The COD of the total and filtered samples, the total suspended solids (TSS), the volatile suspended solids (VSS) and the pH were measured according to the standard methods (APHA, 2005). Determinations of volatile fatty acids (VFA), such as acetic acid (H_{AC}), and bicarbonate alkalinity (BA) followed the methodology described by Dilallo and Albertson (1961) and modified by Ripley et al. (1986). The methylene blue method (method 4500 D) (APHA, 2005) was used to determine the total dissolved sulfide. The sulfate concentrations were measured with a turbidimetric method using the Hach SulfaVer reagent. Influent and effluent samples were collected during alternate cycles. The methane concentrations in the generated biogas were evaluated through gas chromatography (Gow Mac-150) using a thermal conductivity detector (TCD) and Porapack Q column (2 m × ¼ in – 80 to 100 mesh) with a temperature of 35 °C and H_2 as the carrier gas.

3. Results and discussion

After inoculation, the AnSBR was operated for 130 days (65 cycles) under sulfate-reducing conditions characterized by different influent sulfate concentrations (Table 2). Sulfate loading rates (SLRs) were increased from 2.2 to 23.8 g SO_4^{2-}/cycle (Fig. 2).

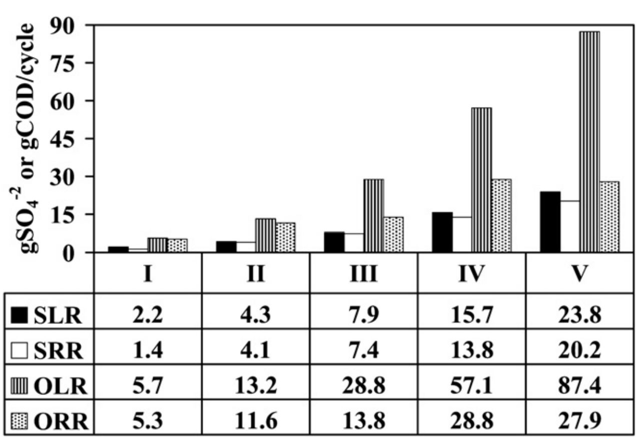

Fig. 2. Mean values of SLR (sulfate loading rate), SRR (sulfate removal rate), OLR (organic loading rate) and ORR (organic removal rate) for several periods of anaerobic batch reactor operation (I—0.25, II—0.5, III—1.0, IV—2.0 and V—3.0 g SO_4^{2-} L^{-1}) in 48 h/cycle.

The maximum sulfate removal rate (SRR) was 20.2 g SO_4^{2-}/cycle at the SLR of 23.8 g SO_4^{2-}/cycle (period V). The sulfate reduction efficiencies reached 99% (Fig. 3) for concentrations of 0.25, 0.5 and 1.0 g SO_4^{2-} L^{-1} (periods I, II and III). The effluent sulfate concentrations remained below 10 mg SO_4^{2-} L^{-1}. Under high sulfate concentrations (2.0 and 3.0 g SO_4^{2-} L^{-1}), the sulfate reduction ranged from 71% to 95% (periods IV and V) (Fig. 3). The average sulfate concentrations in the effluent reactor (Table 3 and Fig. 3) for periods IV and V were 247 and 485 mg L^{-1}, respectively.

A gradual decrease in the organic matter conversion (COD removal) was observed during the experimental phase (Table 3 and Fig. 2). The mean removal efficiencies decreased from 95% to 33% (periods I–V) for organic loading rates (OLR) ranging from 5.7 to 87.4 g COD/cycle (Fig. 2). The mean values of COD_{Total} concentrations were 0.055 g L^{-1} (period I), 0.21 g L^{-1} (period II), 1.99 g L^{-1} (period III), 3.77 g L^{-1} (period IV) and 7.93 g L^{-1} (period V) (Table 3). The mean organic removal rates (ORR) increased as the concentration increased from 0.25 to 2.0 g SO_4^{2-} (5.3–28.8 g COD/cycle) and decreased when the concentration was 3.0 g SO_4^{2-} (27.9 g COD/cycle) (Fig. 2). The decrease in the ORR was probably related to the accumulation of acetic acid (VFA) in the reactor due to inhibition of methanogenesis (Nagpal et al., 2000; Shayegan et al., 2005), which corresponds to low methane concentrations in the biogas. Fig. 4 shows the mean methane and VFA concentrations (such as acetic acid) in several operational periods.

Low substrate removal, VFA accumulation and increased sulfide concentration in the system might be attributed to the inhibition or imbalance of the anaerobic process (Sarti et al., 2010). From period III (1.0 g SO_4^{2-} L^{-1}), a significant concentration of VFA (Fig. 4, Table 3) was generated as a result of the partial oxidation of butanol to acetate. In this case, incompletely oxidizing SRB produced volatile acids, mainly acetic acid (Hansen, 1994; Muyzer and Stams, 2008). Therefore, because VFA was not being consumed by the MPA, the

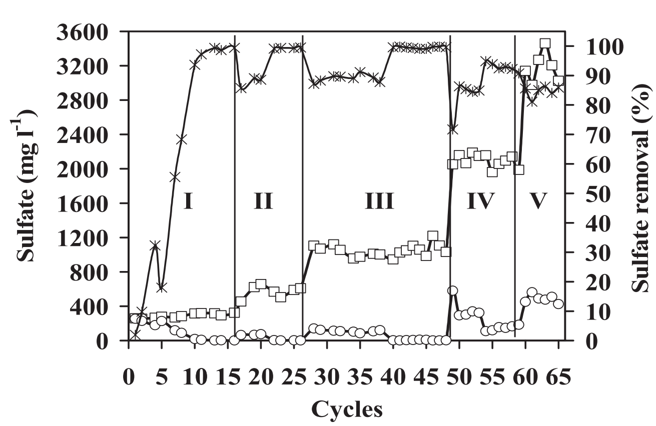

Fig. 3. Temporal variation of sulfate concentration (influent [□] and effluent [○]) and sulfate removal (*) in several periods of anaerobic batch reactor operation.

Table 3
Summary of the average operational parameters (effluent) obtained for the AnSBR. SLR = sulfate removal rate; ORR = organic removal rate.

Variables	Period I	Period II	Period III	Period IV	Period V
Cycle numbers	16	10	22	11	6
SO_4^{2-} (mg L^{-1})	103.6 ± 29.5	31.9 ± 15.2	56.4 ± 29.5	247.8 ± 128.4	485.8 ± 47.4
SRR	1.4	4.1	7.4	13.8	20.2
TDS[a] (mg L^{-1})	1.26 ± 0.30	46.3 ± 9.8	173.5 ± 29.0	189.5 ± 7.8	197.7 ± 6.1
COD_{Total} (mg L^{-1})	55 ± 18	214 ± 50	1994 ± 494	3773 ± 768	7933 ± 408
$COD_{Filtered}$ (mg L^{-1})	28 ± 12	157 ± 55	1904 ± 474	3637 ± 784	7740 ± 426
ORR (g COD_{Total}/cycle)	5.3	11.6	13.8	28.8	27.9
BA (mg $CaCO_3^{-2}$ L^{-1})	188 ± 87	558 ± 41	432 ± 54	631 ± 91	517 ± 66
VFA (mg Hac L^{-1})	24 ± 7.2	32 ± 14	675 ± 99	1322 ± 174	2010 ± 59
pH	6.8–7.8	6.7–7.7	6.5–7.7	6.2–6.8	5.9–6.2
TSS (mg L^{-1})	43 ± 13	30 ± 11	42 ± 11	40 ± 6.0	45 ± 2.0
VSS (mg L^{-1})	31 ± 11	19 ± 6.0	32 ± 10	34 ± 4.0	38 ± 3.0

[a] Total dissolved sulfide.

residual COD in the batch reactor effluent increased (Nagpal et al., 2000), which resulted in the low COD removal efficiency (33%). On the other hand, the bicarbonate alkalinity (BA) generation and the low values of VFA in the previous periods (periods I and II) were considered indicators of the balance between acidogenesis and methanogenesis. The mean VFA values remained between 24 and 32 (periods I and II), while the BA values varied between 188 and 558 mg $CaCO_3$ L^{-1} (Table 3).

The effluent total dissolved sulfide concentrations obtained in this study are shown in Table 3. TDS mean concentrations increased from 1.26 to 197.7 mg L^{-1} in several periods of the batch reactor operation (periods I, II, III, IV and V). The presence of sulfide was particularly critical in period V (3.0 g SO_4^{2-} L^{-1}). It has been reported that the outcome of sulfide inhibition depends not only on the pH, which is directly related to the H_2S concentration, but also on the total dissolved sulfide concentration and the biomass characteristics (O'Flaherty and Colleran, 2000). This finding suggests that both total dissolved sulfide and H_2S may promote an inhibitory effect on the organisms (SRB and MPA). In the batch reactor, there was a decrease in the values (Table 3) of the effluent pH from 6.8 to 6.2 (periods I–IV) and 6.2 to 5.9 in period V, which indicates that the undissociated H_2S was probably the predominant S species. Therefore, the H_2S exerted a greater inhibitory effect on the methanogenic organisms than on the SRB, which was observed at sulfateconcentrationshigherthan2.0g$SO_4^{2-}L^{-1}$(SLR=15.7g$SO_4^{2-}L^{-1}$).

In general, the AnSBR performance was satisfactory because pseudo-steady states were reached in a short period of time after operational changes in sulfate concentration and COD/sulfate ratio occurred, which indicates that significant biomass immobilization was attained on the mineral coal, especially for MPA and SRB (Sarti et al., 2010). Low effluent TSS (30–45 mg L^{-1}) and VSS (19–38 mg L^{-1}) mean concentrations occurred in some experimental periods (Table 3). Therefore, the biomass detachment was reduced in the anaerobic batch reactor. Silva et al. (2006) evaluated the adhesion of SRB and MPA on polyurethane foam, vegetal carbon, low-density polyethylene and alumina-based ceramics in a horizontal flow reactor and found that a different microbial equilibrium was reached in each material; thus, the performance of the reactor was different with each support.

Sarti et al. (2009) have shown that the ratio of electron donors to sulfate feed is important in controlling the relative growth of SRB and MPA, which in turn determines the measure of sulfate reduction and COD removal. If butanol is used as the organic matter (electron donor), then the process must be optimized to operate with the minimum amount of organic carbon necessary for complete sulfate removal. The effect of the COD/sulfate ratio on the sulfate reduction and removal efficiencies was thus assessed by varying the organic carbon or the electron donor. The theoretical optimum is given by the reaction stoichiometry (Eqs. (1) and (2)). In the case of sulfate treatment with butanol, 1.3 g of butanol and 3.4 g of COD are required per gram of sulfate (COD/sulfate = 3.4).

$$SO_4^{2-} + C_4H_9OH \rightarrow 2CH_3COO^- + HS^- + H^+ + H_2O \quad (1)$$

$$C_4H_9OH + 6O_2 \rightarrow 4CO_2 + 5H_2O \quad (2)$$

Fig. 5 shows the plots of COD/sulfate ratios and the maximum value of sulfate removal for several sulfate concentrations (0.25, 0.5, 1.0, 2.0 and 3.0 g SO_4^{2-} L^{-1}) applied to the anaerobic batch reactor. The COD/sulfate ratio in the influent under study was an important controlling parameter for the electron flow to minimize the substrate (butanol) during anaerobic degradation. Both the COD/sulfate

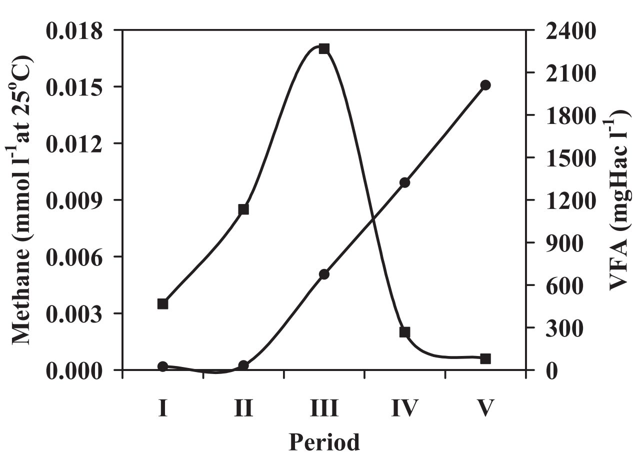

Fig. 4. Mean values of methane (■) and VFA (●) in several periods of anaerobic batch reactor operation.

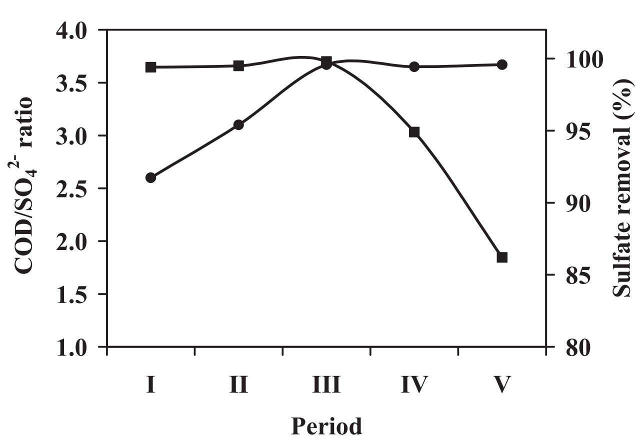

Fig. 5. Mean values of the COD/sulfate ratio (●) and the maximum sulfate removal (■) over several periods of anaerobic batch reactor operation.

ratio and the sulfate concentration can affect the electron flow during sulfate reduction and methanogenesis (Damianovic and Foresti, 2009).

The mean COD/sulfate ratio in the feed varied from 2.6 to 3.67 (Fig. 5, Table 2) during the experimental phase (five periods). These values are near the theoretical value of 3.4 (COD/sulfate ratio based on the stoichiometry) for the reduction of sulfate with butanol (Eqs. (1) and (2)). However, higher sulfate removal efficiencies were achieved at lower influent sulfate concentrations, and sulfate reduction inhibited methanogenesis in this study when the COD/sulfate ratio reached 3.65 and the sulfate concentration was 2.0 g SO_4^{2-} L^{-1} (period IV).

4. Conclusions

The application of butanol (electron donor) for the biological treatment of effluent containing high sulfate concentrations significantly reduced the sulfate concentration. The anaerobic sequential batch reactor filled with mineral coal achieved high sulfate reduction efficiencies (99%) in a short period of operation at different initial sulfate concentrations (0.25–3.0 g SO_4^{2-} L^{-1}).

Based on the results from the AnSBR, it can be concluded that this anaerobic configuration can be used for the combined removal of sulfate and organic matter at sulfate influent concentrations below 1.0 g SO_4^{2-} L^{-1} if butanol is used as the electron donor. At influent sulfate concentrations higher than 1.0 g SO_4^{2-} L^{-1}, high VFA concentrations (residual COD) and sulfur-reduced compounds (TDS or H_2S) were generated. In this case, simultaneous methanogenesis and sulfidogenesis suppression were observed.

Current studies debate the value of the COD/sulfate ratio required for sulfate biological treatment; however, the predominance of sulfate reduction over methanogenesis with butanol was observed when the COD/sulfate ratio was 3.65 and the sulfate concentration was 2.0 g SO_4^{2-} L^{-1} (period IV). The results indicate the possibility of using butanol in anaerobic reactors as the electron donor for sulfate reduction. However, both sulfate and organic matter were removed most efficiently when the COD/sulfate ratios were below 3.67.

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