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AnSBBR Applied to Organic Matter and Sulfate Removal: Interaction Effect Between Feed Strategy and Cod/Sulfate Ratio

Gregor F. Friedl · Gustavo Mockaitis · José A. D. Rodrigues · Suzana M. Ratusznei · Marcelo Zaiat · Eugênio Foresti

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Abstract A mechanically stirred anaerobic sequencing batch reactor containing anaerobic biomass immobilized on polyurethane foam cubes, treating low-strength synthetic wastewater (500 mg COD L^{-1}), was operated under different operational conditions to assess the removal of organic matter and sulfate. These conditions were related to fill time, defined by the following feed strategies: batch mode of 10 min, fed-batch mode of 3 h and fed-batch mode of 6 h, and COD/[SO_4^{2-}] ratios of 1.34, 0.67, and 0.34 defined by organic matter concentration of 500 mg COD L^{-1} and sulfate concentrations of 373, 746, and 1,493 mg SO_4^{2-} L^{-1} in the influent. Thus, nine assays were performed to investigate the influence of each of these parameters, as well as the interaction effect, on the performance of the system. The reactor operated with agitation of 400 rpm, total volume of 4.0 L, and treated 2.0 L synthetic wastewater in 8-h cycles at 30±1°C. During all assays, the reactor showed operational stability in relation to the monitored variables such as COD, sulfate, sulfide, sulfite, volatile acids, bicarbonate alkalinity, and solids, thus demonstrating the potential to apply this technology to the combined removal of organic matter and sulfate. In general, the results showed that the 3-h fed-batch operation with a COD/[SO_4^{2-}] ratio of 0.34 presented the best conditions for organic matter removal (89%). The best efficiency for sulfate removal (71%) was accomplished during the assay with a COD/[SO_4^{2-}] ratio of 1.34 and a fill time of 6 h. It was also observed that as fill time and sulfate concentration in the influent increased, the ratio between removed sulfate load and removed organic load also increased. However, it should be pointed out that the aim of this study was not to optimize the removal of organic matter and sulfate, but rather to analyze the behavior of the reactor during the different feed strategies and applied COD/[SO_4^{2-}] ratios, and mainly to analyze the interaction effect, an aspect that has not yet been explored in the literature for batch reactors.

G. F. Friedl · G. Mockaitis · M. Zaiat · E. Foresti
Departamento de Hidráulica and Saneamento, Escola de Engenharia de São Carlos, Universidade de São Paulo (SHS/EESC/USP), Av. Trabalhador São-Carlense 400, CEP 13.566-590 São Carlos, SP, Brasil

J. A. D. Rodrigues (✉) · S. M. Ratusznei
Escola de Engenharia Mauá, Instituto Mauá de Tecnologia (EEM/IMT), Praça Mauá 1, CEP 09.580-900 São Caetano do Sul, SP, Brasil
e-mail: rodrigues@maua.br

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Nomenclature

$ASOL_{ST}$	Applied specific load of unfiltered organic matter [(mg COD (L day)$^{-1}$]
ASSL	Applied specific sulfate load [mg COD (L day)$^{-1}$]
$AVOL_{ST}$	Applied volumetric load of unfiltered organic matter [g COD (L day)$^{-1}$]
AVSL	Applied volumetric sulfate load [g COD (L day)$^{-1}$]
BA	Bicarbonate alkalinity (mg $CaCO_3$ L^{-1})
C_{H2S}	Concentration of dissolved hydrogen sulfite (H_2S) (mgH_2S L^{-1})
C_{HS}	Concentration of sulfide in the form of dissolved HS^- (mg HS^- L^{-1})
COD	Chemical oxygen demand
C_{SF}	Concentration of filtered oxidizable matter (mg COD L^{-1})
C_{SO3}	sulfite concentration in the effluent (mg SO_3^{2-} L^{-1})
C_{SO4}	Sulfate concentration in the effluent (g SO_4^{2-} L^{-1})
C_{SO4}^{in}	Sulfate concentration in the influent (g SO_4^{2-} L^{-1})
C_{SOF}	Concentration of filtered organic matter (mg COD L^{-1})
C'_{SOF}	Concentration of filtered organic matter in the effluent [g COD (L d)$^{-1}$]
C_{SOT}	Concentration of total organic matter (mg COD L^{-1})
C_{ST}	Concentration of total oxidizable matter (mg COD L^{-1})
C_{ST}^{in}	Concentration of organic matter in the influent (g COD L^{-1})
C_{TDS}	Concentration of total dissolved sulfide (mg TDS L^{-1})
C_{XTS}'	Specific concentration of total solids in the polyurethane foam (gST gEPU^{-1})
C_{XTVS}	Biomass concentration in the reactor (g TVS L^{-1})
C_{XTVS}'	Specific concentration of total volatile solids in the polyurethane foam (g TVS g EPU^{-1})
EPU	Mass of the polyurethane foam (g EPU)
N	Number of cycles per day (cycles day^{-1})
pKa	Equilibrium constant of HS^-/H_2S (7,02)
$RSOL_{SOF}$	Removed specific load of filtered organic matter [mg COD (g TVS day)$^{-1}$]
RSSL	Removed specific sulfate load [mg COD (g TVS day)$^{-1}$]
$RVOL_{SOF}$	Removed volumetric load of filtered organic matter [g COD (L day)$^{-1}$]
RVSL	Removed volumetric sulfate load [g COD (L d)$^{-1}$]
TS	Total solids (mg L^{-1})
TVA	Total volatile acids (mg HAc L^{-1})
TVS	Total volatile solids (mg L^{-1})
V_A	Volume fed per cycle (L cycle^{-1}))
V_R	working volume of the reactor (L)

Introduction

Sulfate (SO_4^{2-}), the most stable ion of sulfur, occurs in nature, for example, from volcanic activities, acid rock drainage, and in seawater. Anthropogenic activities that contribute to sulfate pollution of natural water bodies include industrial activities, for instance wood and pulp processing, food production, processing of xenobiotics, and utilization of fossil fuels

[1]. The toxicity of sulfate arises from the inhibitory effects of sulfide hydrogen, the end product of the sulfate reduction process, on microorganisms responsible for the degradation of organic matter. Under aqueous anaerobic conditions, sulfate can be used as an electron receptor in the oxidation process of organic matter by a group of specialized bacteria, i.e., sulfate-reducing bacteria (SRB), to produce sulfide ion. Most of the SRB are mesophilic bacteria having optimum growth rates at ambient temperature around 33°C. Examples of electron donors are hydrogen, lactate, propionate, acetate, ethanol, and other alcohols. The SRB can be divided into two groups: one that completely oxidizes the substrate to carbon dioxide and the other that oxidizes organic matter partly to acetate.

Conventional technologies used to treat wastewater contaminated with sulfate, such as reverse osmosis or precipitation through barium sulfate or barium carbonate, are expensive and produce solid chemical residues. Owing to advances in anaerobic wastewater treatment, the treatment in anaerobic reactors has become an economically and technically feasible alternative. Usually, the biological reduction of sulfate in anaerobic digesters is considered undesirable, since the presence of sulfate favors the growth of SRB, which compete with methanogenic microorganisms for disposable substrates. The relation between organic matter (COD) and sulfate concentration [SO_4^{2-}] is, therefore, an important aspect for the operation of anaerobic systems. Based on the stoichiometry of sulfate reduction, Lens et al. [2] established a theoretical relationship between COD (electron donor) and sulfate concentration (electron acceptor). With a COD/[SO_4^{2-}] ratio of 0.67 theoretically all the organic matter can be oxidized through sulfate reduction, in other words, theoretically all the sulfate present can be reduced to sulfide.

There are several studies regarding the factors affecting the performance of continuous anaerobic reactors in sulfate removal. However, studies regarding the feed strategy in discontinuous reactors, one of the main characteristics of the system used in this work, are still rare in the literature. Within this context, this work aims to investigate the behavior of a mechanically stirred AnSBBR with 8-h cycle length, equipped with a draft tube, containing biomass immobilized on polyurethane foam, treating synthetic wastewater with different sulfate concentrations (373, 746, and 1,493 mg SO_4^{2-} L^{-1}) and low organic matter concentration (500 mg COD L^{-1}) and operating at different fill modes (batch/fed-batch). In this way, both the influence of feed strategy and of the COD/[SO_4^{2-}] ratio (1.34, 0.67, and 0.34) were studied by using different fill times (10-min batch; 3-h and 6-h fed-batch) and implementing different sulfate concentrations in the influent at constant organic matter concentration.

The interaction effects between these variables on the stability and efficiency of organic matter and sulfate removal were also analyzed, enabling to expand our knowledge of the performance of sequencing batch reactors in the combined removal of organic matter and sulfate, aiming at potential applications on industrial scale.

Materials and Methods

Experimental Setup

The scheme of the reactor used in this study, with configuration based on the reactor used by Ratusznei et al. [3], is shown in Fig. 1. The volume occupied by the biomass immobilized on polyurethane foam was 0.5 L, and the volume of the residual liquid in the reactor was 1.5 L. The volume fed and discharged per cycle using diaphragm pumps were both 2.0 L, and the working volume of the reactor was 4.0 L.

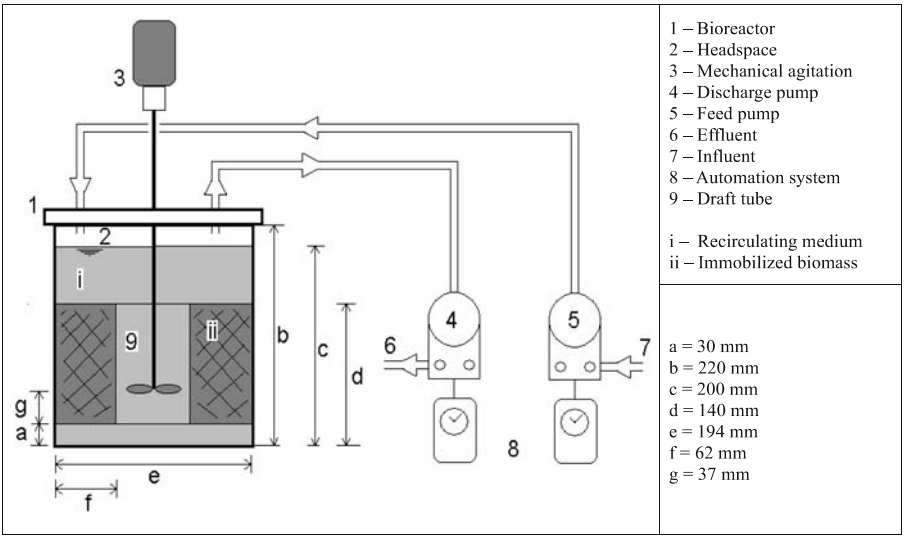

Fig. 1 Anaerobic bioreactor with mechanical agitation provided by a helix propeller and equipped with a draft-tube, containing immobilized biomass

Bioreactor operation was carried out at a constant temperature of 30±1°C, guaranteed by placing the reactor in a temperature controlled chamber. The agitation frequency was set at 400 rpm, implemented by a helix propeller (inclination of 45° of the blades) in a draft-tube, a value defined in the work of Michelan et al. [4].

Inoculum and Inert Support

The inoculum came from a UASB reactor treating wastewater from a poultry slaughterhouse. The sludge had an initial concentration of total solids (TS) and total volatile solids (TVS) of 64.1 g TS L^{-1} and 52.8 g TVS L^{-1}, respectively.

As support for immobilization of the biomass, 5-mm polyurethane foam cubes (EPU) with an apparent density of 23 kg m^{-3} and porosity near 95% were used. Inoculation was performed according to the method proposed by Ratusznei et al. [5] using the sludge from the UASB reactor-treating wastewater from a poultry slaughterhouse. The sludge was crushed through a 0.5-mm mesh nylon sieve, resulting in a suspension in which the foam was completely immersed. Intense homogenization followed after which 2-h rest was allowed. Poorly adhered solids were washed off, and the medium was drained. After this period the foam with the immobilized biomass was placed in the reactor. Next, to wash out the weakly attached cells, the reactor was fed with the same wastewater used during the experiments. The wastewater was discharged, and the inoculum was ready for use in the reactor.

Synthetic Wastewater

The synthetic wastewater used in this study with 500 mg COD L^{-1} contained: carbohydrates (sucrose 35 mg L^{-1}; starch 114 mg L^{-1}), proteins (meat extract 208 mg L^{-1}), lipids (soybean oil 51 mg L^{-1}), and micronutrients (NaCl 250 mg L^{-1}, $MgCl_2 \cdot 6H_2O$ 7.5 mg L^{-1},

$CaCl_2 \cdot 2H_2O$ 4.5 mg L^{-1}), and also $NaHCO_3$ (200 mg L^{-1}), used as buffer. Three drops per liter of commercial detergent were also added to emulsify the soybean oil. The synthetic wastewater was sterilized (121°C for 15 min) to guarantee the characteristics of the wastewater throughout the operation of the reactor. This procedure was adopted because, once a day, the bottle containing synthetic wastewater was changed for another one containing fresh wastewater. So, sterilization was used to prevent contamination during this period, maintaining concentration levels (mainly COD and sulfate). It should be mentioned that the sterilization procedure performed in this work did not modify the physical–chemical characteristics of the synthetic wastewater and only prevented contamination by undesirable microorganisms.

Sulfate in the form of sodium sulfate (Na_2SO_4) was supplemented to the wastewater at the following concentrations based on the COD/[SO_4^{2-}] ratios used in this study: 552, 1,103, and 2,207 mg Na_2SO_4 L^{-1} for COD/[SO_4^{2-}] ratios of 1.34, 0.67, and 0.34, respectively. This means that for a constant organic matter concentration of 500 mg COD L^{-1} in the wastewater influent, concentrations of 373, 746, and 1,493 mg SO_4^{2-} L^{-1} were added, respectively.

The justification of the synthetic wastewater used is based on the fact that some chemical industries generate wastewater containing different levels of sulfate in a discontinuous way, and these effluents are frequently generated in amounts that do not justify a continuous wastewater plant. For these industries, an AnSBBR presents economical advantages. Within this context, considering that the main objective of this work was to study the influence of different sulfate concentrations and low organic matter concentration, i.e., COD/[SO_4^{2-}] ratio, using different fill times, the potential practical application of the results obtained here is the treatment of wastewaters that might need low amounts of organic matter supplementation. The purpose of this procedure was to simulate carbon source supplementation to a synthetic wastewater, with composition (excepting sulfate) close to that of a domestic wastewater [6], using a cheap and readily degradable compound.

Theoretical Fundamentals

Analysis of the results from anaerobic reactors employed in sulfate removal requires calculation of some important variables that take into account the products of the sulfidogenesis. Since, usually the levels of inorganic oxidizable matter are low and hardly affect the result of the analysis, it was convenient to assume the COD value as a measure of the oxidizable organic matter. However, in systems where concentrations of sulfide (C_{TDS}), sulfite (C_{SO3}), and other not totally oxidized inorganic species are significant, it is necessary to consider the presence of these substances in the calculation of oxidizable organic matter concentration for unfiltered and filtered samples (C_{ST} and C_{SF}). The concentrations of organic matter for unfiltered and filtered samples can thus be determined (C_{SOT} and C_{SOF}), as shown in the following equations:

$$C_{SOT} = C_{ST} - (2.C_{TDS} + 1/5 \times C_{SO3}) \quad (1)$$

$$C_{SOF} = C_{SF} - (2 \times C_{TDS} + 1/5 \times C_{SO3}) \quad (2)$$

The concentration of sulfide found in the samples corresponds to the sum of the forms of sulfide present (HS^-, H_2S, and S^{2-} dissolved) and will be called total dissolved sulfide (C_{TDS}). The concentration of the anions in the sample is calculated according to the

chemical equilibrium of those ions in a relation of dependency to the pH (and p*K*a). Nevertheless, as shown by Lens et al. [2], the S^{2-} ion is not present in the pH range where anaerobic digestion occurs. So, the concentration of free sulfide hydrogen (C_{H2S}) can be calculated by the following equation [7]:

$$C_{H2S} = \frac{C_{TDS}}{1 + 10^{pH - pKa}} \tag{3}$$

The sulfide concentration in the form of HS^- (C_{HS}) can be calculated according to:

$$C_{HS} = C_{TDS} - C_{H2S} \tag{4}$$

The applied or removed volumetric load, which represents the quantity of applied or removed organic matter or sulfate during a specific period per volume unit of the reactor, was calculated for filtered and unfiltered samples, as shown below. The values of the specific loads were also calculated, where the volumetric load is expressed per biomass concentration in the reactor (C_{XTVS} in g TVS L^{-1}).

$$AVOL_{ST} = \frac{C_{ST}^{in} \times V_A \times N}{V_R} \tag{5}$$

$$AVSL = \frac{C_{SO4}^{in} \times V_A \times N}{V_R} \tag{6}$$

$$ASOL_{ST} = \frac{C_{ST}^{in} \times V_A \times N}{V_R \times C_{XTVS}} \tag{7}$$

$$ASSL = \frac{C_{SO4}^{in} \times V_A \times N}{V_R \times C_{XTVS}} \tag{8}$$

$$RVOL_{SOF} = \frac{\left(C_{ST}^{in} - C_{SOF}\right) \times V_A \times N}{V_R} \tag{9}$$

$$RVSL = \frac{\left(C_{SO4}^{in} - C_{SO4}\right) \times V_A \times N}{V_R} \tag{10}$$

$$RSOL_{SOF} = \frac{\left(C_{ST}^{in} - C_{SOF}\right) \times V_A \times N}{V_R \times C_{XTVS}} \tag{11}$$

$$RSSL = \frac{\left(C_{SO4}^{in} - C_{SO4}\right) \times V_A \times N}{V_R \times C_{XTVS}} \tag{12}$$

Physicochemical and Microbiological Analyses

Analyses of the influent and effluent operational variables were carried out in accordance with the APHA [8] considering: total oxidizable matter of the filtered (C_{SF}) and unfiltered samples (C_{ST})—colorimetric method 5220 D; total solids, total suspended solids, total volatile solids, and volatile suspended solids (TS, TSS, TVS, VSS)—methods 2540 B (total solids dried at 103–105°C), 2540 D (total suspended solids dried at 103–105°C), and 2540 E (fixed and volatile solids ignited at 550°C); sulfate (SO_4^{2-})—turbidimetric method 4500-SO_4^{2-} E; total dissolved sulfide (TDS)—methylene blue method 4500-S^{2-} D; and sulfite (SO_3^{2-})—iodometric method 4500-SO_3^{2-} B. The titration method proposed by Dilallo and Albertson [9] modified by Ripley et al. [10] was also considered in the determination of alkalinity (total, partial, intermediate, and bicarbonate).

Chromatographic analyses were carried out with a Hewlett Packard® model 6890 gas chromatograph to quantify composition of the biogas (CH_4 and CO_2) and of the intermediate volatile acids (acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric). Biogas composition was analyzed using a thermal conductivity detector, HP-porapak Q (80/100 mesh) column (length 6 ft; column OD 0.125 inch) and sample volume of 1 mL; drag gas was hydrogen at a flow rate of 50.0 mL/h, the column, injector, and detector temperatures were 35°C, 60°C, and 160°C, respectively. Intermediate volatile acid samples were analyzed using a flame ionization detector at 300°C, an HP-innowax column (crosslinked polyurethane glycol—film thickness 0.25 μm; length 30 m; phase ratio 250; column ID 0.25 mm) and sample volume of 1 μL. The injector temperature was kept at 250°C; the oven was held at 100°C for 3 min, after which it was heated at a rate of 5°C min^{-1} to 180°C and kept at that temperature for 5 min.

At the end of the assays after 302 days of operation, the biomass in the polyurethane foam was quantified in terms of total volatile solids (TVS), obtaining the amount of biomass present in the reactor (X_{TVS}), the concentration (C_{XTVS}), and the specific concentration (C_{XTVS}'), in terms of total volatile solids mass (gTVS) per polyurethane foam mass (gEPU). The solids adhered to the polyurethane foam were determined by removing the biomass with distilled water from approximately four cubes of the support. The washed foam cubes were then dried at 105°C for 24 h to determine the dry weight. The detached solids were quantified in accordance with the conventional methods in the APHA [8].

Microbiological analysis of the anaerobic sludge was carried out by common optical and fluorescence phase contrast microscopy, using a BX41 Olympus® microscope. A preliminary characterization of the microbiological communities present was performed in accordance with Holt et al. [11].

Experimental Procedure

The feed strategies, using a treatment cycle of 8 h, were batch mode (fill time of 10 min—conditions B) and fed-batch mode (fill time of 3 h—conditions FB1; and 6 h—conditions FB2). The different COD/[SO_4^{2-}] ratios applied were 1.34, 0.67, and 0.34 (conditions I, II, and III, respectively), maintaining the organic matter concentration of the synthetic wastewater at 500 mg COD L^{-1}. The experiments were initiated by operating the bioreactor for each assay for the proposed period and varying the COD/[SO_4^{2-}] ratio and the fill time (Table 1).

During the 27-day preliminary assay, the following were observed: reactor startup, adaptation of the biomass, and adaptation of the analytical methods utilized in this study. During this assay, the reactor was fed as described under condition I-B (batch mode) with an influent COD/[SO_4^{2-}] ratio of 1.34.

Table 1 Summary of the performed experiments[a].

Experimental conditions		Feed strategy		
		Batch (B) Feed time of 10 min	Fed-Batch 1 (FB1) Feed time of 3 h	Fed-Batch 2 (FB2) Feed time of 6 h
COD/[SO_4^{2-}] ratio	1.34	I-B (42 days)	I-FB1 (30 days)	I-FB2 (36 days)
	0.67	II-B (34 days)	II-FB1 (22 days)	II-FB2 (35 days)
	0.34	III-B (27 days)	III-FB1 (27 days)	III-FB2 (22 days)

C_{SO4}^{in}: 373, 746, and 1,493 mg $SO_4^{2-}.L^{-1}$ for COD/[SO_4^{2-}] ratio of 1.34, 0.67, and 0.34, respectively; Total cycle time: 8 h; Temperature: 30±1°C; Total liquid volume in the reactor: 4.0 ; Volume fed and discharged per cycle: 2.0 L; Agitation: 400 rpm (helix propeller with inclination of 45° of the blades in a draft-tube); C_{XTVS}': 1.3 $g_{TVS}.g_{EPU}^{-1}$; C_{XTS}': 1.4 $g_{ST}.g_{EPU}^{-1}$; C_{XTVS} 18.0 g TVS L^{-1} (Approximately 82.1 g total volatile solids in the reactor and considering 4.0 L working volume in the reactor)

[a] C_{ST}^{in}: 500 mg COD L^{-1}

In the first three assays (I-B, I-FB1 and I-FB2, in Table 2), the reactor operated at the three proposed feed strategies and with a COD/[SO_4^{2-}] ratio of 1.34. The purpose of these assays was to compare the operational results with regard to efficiency (organic matter and sulfate removal) and stability at the different feed strategies. The objective of the other six assays with COD/[SO_4^{2-}] ratios of 0.67 (II-B, II-FB1, and II-FB2, in Table 1) and 0.34 (III-B, III-FB1, and III-FB2, in Table 2) was to compare the effect of the COD/[SO_4^{2-}] ratio in each feed mode, as well as to evaluate the most efficient feed strategy.

In each assay, after having attained operation stability, i.e., approximately constant values of the monitored values, profiles were run over the course of the operation cycle and the following were quantified: substrate concentration from filtered samples (COD), sulfate

Table 2 Summary of the main influent and effluent monitored variables[a].

Condition	Effluent					
	C_{SOF}	C_{SO4}	COD/[SO_4^{2-}]	C_{TDS}	TVA	BA
I-B	68±17$^{(20)}$	293±34$^{(14)}$	0.30±0.14$^{(14)}$	9±4$^{(13)}$	21±6$^{(15)}$	379±68$^{(15)}$
I-FB1	117±29$^{(16)}$	190±54$^{(14)}$	0.82±0.39$^{(14)}$	24±10$^{(11)}$	29±7$^{(12)}$	562±37$^{(12)}$
I-FB2	119±30$^{(16)}$	113±38$^{(21)}$	1.08±0.77$^{(21)}$	39±13$^{(9)}$	38±15$^{(9)}$	460±33$^{(9)}$
II-B	138±46$^{(16)}$	475±85$^{(15)}$	0.17±0.17$^{(15)}$	48±22$^{(10)}$	64±6$^{(9)}$	511±78$^{(9)}$
II-FB1	150±29$^{(10)}$	442±42$^{(12)}$	0.43±0.06$^{(12)}$	67±9$^{(5)}$	55±14$^{(6)}$	556±53$^{(6)}$
II-FB2	128±53$^{(14)}$	448±28$^{(14)}$	0.35±0.11$^{(14)}$	52±15$^{(8)}$	35±11$^{(11)}$	560±50$^{(11)}$
III-B	155±20$^{(9)}$	1,211±119$^{(12)}$	0.15±0.03$^{(12)}$	60±15$^{(6)}$	54±5$^{(8)}$	565±80$^{(8)}$
III-FB1	55±18$^{(10)}$	1,210±77$^{(12)}$	0.08±0.03$^{(12)}$	87±7$^{(7)}$	26±5$^{(7)}$	611±25$^{(7)}$
III-FB2	135±14$^{(10)}$	1,216±73$^{(10)}$	0.15±0.02$^{(10)}$	59±6$^{(7)}$	47±10$^{(6)}$	536±34$^{(6)}$
Influent						
I	502±27$^{(43)}$	385±35$^{(46)}$	1.32±0.14$^{(38)}$	–	39±7$^{(29)}$	192±27$^{(29)}$
II	503±32$^{(38)}$	752±38$^{(35)}$	0.67±0.04$^{(33)}$	–	40±7$^{(23)}$	184±36$^{(23)}$
III	505±22$^{(29)}$	1,529±83$^{(31)}$	0.30±0.10$^{(30)}$	–	38±5$^{(20)}$	195±14$^{(20)}$

C_{SOF}/C_{ST} mg COD.L^{-1}; C_{SO4} mg SO_4^{2-} L^{-1}; C_{TDS} mg TDS L^{-1}, *TVA* mg HAc L^{-1}: BA mg$CaCO_3$ L^{-1}.

[a] Values between parentheses refer to the number of samples analyzed in the measurement.

concentration, bicarbonate alkalinity, total and intermediate volatile acids, methane, sulfite and sulfide concentration, pH, dissolved oxygen, and redox potential. These profiles led to a better understanding of the degradation routes over the course of the cycle.

Results and Discussion

First of all, it should be mentioned that the main objective of this work was to study the AnSBBR applied to the treatment of synthetic wastewater with different sulfate concentrations (373, 746, and 1,493 mg $SO_4^{2-}L^{-1}$) and low organic matter concentration (500 mg COD L^{-1}) operating at different fill modes (batch/fed-batch). The focus was not to achieve the best condition but rather to analyze the influence of feed strategy (fill times of 10-min batch; 3-h and 6-h fed-batch) and of COD/[SO_4^{2-}] ratio (1.34, 0.67, and 0.34) on the stability and removal efficiency of organic matter and sulfate as well as the interaction effects between these variables. Therefore, the best condition was not mentioned at all, at least in an explicit way

During the 27-day preliminary assay, there was little initial activity of the SRB and, therefore, low sulfate production. Significant values of sulfide concentration were observed at day 24, and at this point, the assays were initiated. Results are presented in Tables 2 and 3 and Figs. 2, 3, 4, 5, and 6, showing the average values of the monitored variables over the course of the nine assays. From these tables, and figures it was possible to interpret the relation between conditions I (COD/[SO_4^{2-}] of 1.34), II (COD/[SO_4^{2-}] of 0.67), and III (COD/[SO_4^{2-}] of 0.34) and the feed strategies B (feeding time of 10 min), FB1 (feeding time of 3 h), and FB2 (feeding time of 6 h). A summary of the main results obtained in the investigated conditions is shown in Tables 2 and 3, presenting average values with respective standard deviations; values between parentheses refer to the number of samples analyzed in the measurement.

Organic Matter Removal

Figure 2 contains the organic matter concentrations and the respective removed loads of filtered organic matter. It can be seen that during the assays with a COD/[SO_4^{2-}], ratio of

Table 3 Summary of the applied and removed loads.

		COD/[SO_4^{2-}] ratio								
		1.34			0.67			0.34		
		B	FB1	FB2	*B*	FB1	FB2	*B*	FB1	FB2
$AVOL_{ST}$	[g COD.(L day)$^{-1}$]		0.75			0.76			0.76	
AVSL	[g SO_4^{2-}.(L day)$^{-1}$]		0.58			1.13			2.29	
$ASOL_{ST}$	[mg COD (g TVS day)$^{-1}$]		42			42			42	
ASSL	[mgSO_4^{2-}.(g TVS day)$^{-1}$]		32			63			127	
$RVOL_{SOF}$	[g COD.(L day)$^{-1}$]	0.64	0.58	0.59	0.56	0.53	0.56	0.52	0.68	0.56
RVSL	[g SO_4^{2-}.(L day)$^{-1}$]	0.13	0.30	0.41	0.40	0.48	0.46	0.41	0.49	0.55
$RSOL_{SOF}$	[mg COD.(g TVS day)$^{-1}$]	36	32	33	31	29	31	29	38	31
RSSL	[mg SO_4^{2-}.(g TVS day)$^{-1}$]	7	17	23	22	27	25	22	27	30

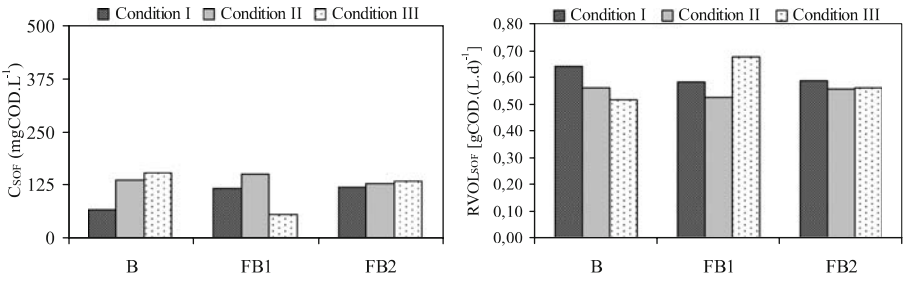

Fig. 2 Concentration (C_{SOF}) and removed volumetric load ($RVOL_{SOF}$) of filtered organic matter during conditions I, II, and III and feed strategies B, FB1, and FB2

1.34 (conditions I) there was improved organic matter removal for the batch assays (I-B) and with increasing fill time (conditions I-FB1 and I-FB2) this removal decreased. During the assays with COD/[SO_4^{2-}] ratios of 0.67 and 0.34 (conditions II and III, respectively) opposite behavior was observed in organic matter removal. Similar to the results obtained under condition I (COD/[SO_4^{2-}] ratio of 1.34) batch feeding (conditions II-B and III-B) represented an unfavorable feed strategy for the assimilation of the substrate compared to the fed-batch feeding (conditions II-FB1, II-FB2, III-FB1 and III-FB2). Nevertheless, the activity of the SRB and the consequent production of sulfide were high enough to induce a possible inhibitory effect on the methane forming process, resulting in increased accumulation of volatile acids during the batch assays (conditions II-B and III-B, in Fig. 4). Increasing the feed time (conditions II-FB1, II-FB2, III-FB1, and III-FB2), the activity of the SRB increased and contributed to increased consumption of acetate by these microorganisms. As a consequence, the efficiency of organic matter removal was improved during the fed-batch assays (conditions II-FB1, II-FB2, III-FB1, and III-FB2) in relation to the batch assays (conditions II-B and III-B).

Sulfate Removal

Figure 3 contains the sulfate concentrations in the influent and effluent as well as the applied and removed sulfate loads. It can be observed that during condition I (COD/[SO_4^{2-}] ratio of 1.34), the sulfate removal was three times as high at condition I-FB2 as at condition I-B, whereas at conditions II and III (COD/[SO_4^{2-}], ratio of 0.67 and 0.34, respectively), there is no feed strategy that significantly favors or disfavors the SRB. Moreover, from this figure, it can be concluded that the sulfate removal efficiencies at the respective feed

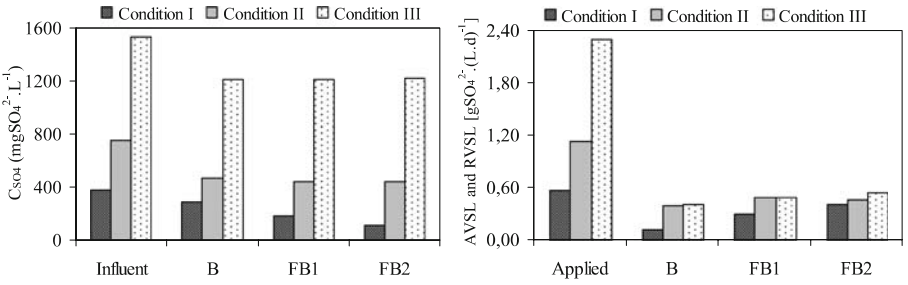

Fig. 3 Sulfate concentration (C_{SO4}) in the influent and effluent, applied (AVSL) and removed (RVSL) volumetric sulfate load at conditions I, II, and III and feed strategies B, FB1, and FB2

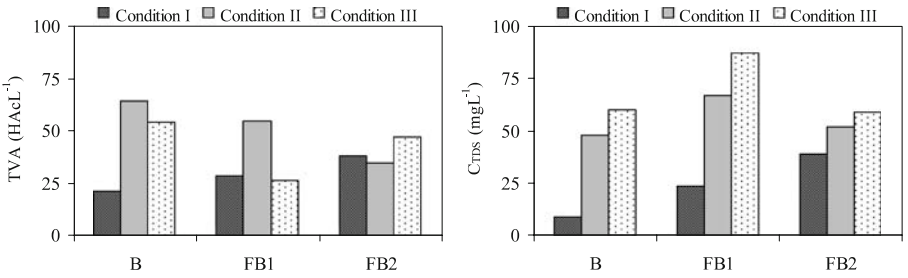

Fig. 4 Total volatile acids (TVA) and total dissolved sulfide (C_{TDS}) in the effluent under conditions I, II, and III and feed strategies B, FB1, and FB2

strategies were higher when sulfate concentrations in the influent were lower, with the exception of the low SRB activity at condition I-B. Therefore, in the assays under condition I (COD/[SO_4^{2-}] ratio of 1.34), a higher sulfate removal efficiency was observed compared to the assays with the same feed strategy but with higher sulfate concentrations in the influent, that is, during the assays under conditions II and III (COD/[SO_4^{2-}] ratios 0.67 and 0.34, respectively). This seems to be related to the difficulty of the SRB to utilize the organic matter that remained as residual substrate in the reactor (Fig. 2), which also contributed to lower sulfate removal during the assays under conditions II and III, as theoretically expected. The removed sulfate loads (RVSL) ranged from 0.30 g SO_4^{2-} (L days)$^{-1}$ (condition I-FB1) to 0.55 g SO_4^{2-} (L days)$^{-1}$ (condition III-FB2).

Sulfide Production and Consumption

Figure 4 shows that the sulfide production increased at the respective feed strategies as the COD/[SO_4^{2-}] ratio decreased (this is, from condition I to condition III), as a consequence of the increased sulfate concentration. However, it can be seen from the same figure that condition FB1 (fill time of 3 h) favored sulfite production at conditions II and III, this is, in the situation where the COD/[SO_4^{2-}] ratio was equal and below the stoichiometric value (0.67 and 0.34, respectively), demonstrating the effect of fill time on sulfidogenesis.

Furthermore, it can be seen that during the assays under condition I (COD/[SO_4^{2-}] ratio of 1.34), the increase in feed time (this is, from condition B to condition FB2) contributed to a loss in competitiveness of the methanogenic archaea in relation to the sulfidogenic

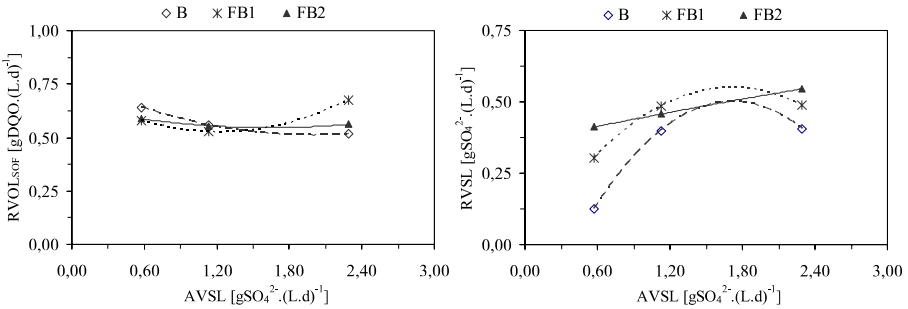

Fig. 5 Removed volumetric load of filtered organic matter ($RVOL_{SOF}$) and removed volumetric sulfate load (RVSL) as a function of the applied volumetric sulfate load (AVSL) under conditions I, II, and III and feed strategies B, FB1, and FB2

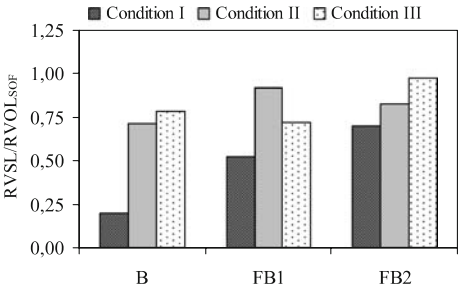

Fig. 6 Ratio between removed volumetric sulfate load (RVSL) and removed volumetric load of filtered organic matter ($RVOL_{SOF}$) under conditions I, II, and III and feed strategies B, FB1, and FB2

bacteria, followed by an increased accumulation of volatile acids. However, during the assay under conditions II and III (COD/$[SO_4^{2-}]$ ratios of 0.67 and 0.34, respectively), an opposite trend was observed; this is reduced accumulation of volatile acids with increasing fill time (this is, from condition B to condition FB2). There was also increased bicarbonate alkalinity production during the fed-batch assays (conditions FB1 and FB2), in which the SRB were more active. COD/$[SO_4^{2-}]$ ratio seemed to affect the production of alkalinity especially during the batch mode operations (conditions B), in which the production of bicarbonate alkalinity increased with decreasing COD/$[SO_4^{2-}]$ ratio. This behavior was probably induced by the growing activity of the SRB under conditions II-B and III-B and by a decrease in bicarbonate consumption due to methanogenesis over the course of these assays.

Analyzing the primary potential inhibition agent in the reactor, i.e., sulfide, the total dissolved sulfide (TDS) shown in Table 2 was higher under condition III (from 59 to 87 and 59 mg TDS L^{-1}) compared with conditions II (from 48 to 67 mg TDS L^{-1}) and I (from 9 to 39 mg TDS L^{-1}). These results are proportional to the respective higher value of the influent and seem to indicate that the presence of sulfide did not cause inhibition. Rinzema and Lettinga [12] state that the concentration of hydrogen sulfide in the anaerobic bioreactor should never exceed the critical value of 150 mg L^{-1}; O'Flaherty et al. [13] observed methanogenesis inhibition at concentrations of total sulfide varying from 300 to 1,579 mg L^{-1} (110 to 243 mg H_2S L^{-1}), in the pH range from 6.5 to 8.0, when ethanol was used as carbon source (COD/sulfate equal to 3); and Isa et al. [14] observed inhibition of 50% of the methanogenic microorganisms only when H_2S levels exceeded 1,000 mg L^{-1}.

Fill time and COD/$[SO_4^{2-}]$ Ratio Interaction Effects

Figure 5 shows that in the assays with 10-min feed time (conditions B) and during assays with 3-h feed time (conditions FB1), the increase in applied volumetric sulfate load (AVSL) did not induce improved sulfate removal, whereas during the assays with 6-h fill time (conditions FB2), the removed volumetric sulfate load (RVSL) increased linearly as the applied volumetric sulfate load (AVSL) increased. With regard to the batch assays (conditions B), a possible cause for the stagnation of the removal could be the disadvantage of the SRB in the competition for substrate in this feed strategy. However, during the assays with feeding time of 3 h (conditions FB1) sulfate removal, in terms of removed volumetric sulfate load (RVSL), did not follow the organic matter removal (see also Fig. 2) as a function of the increase in applied volumetric sulfate load (AVSL). Verification of the influence of the COD/$[SO_4^{2-}]$ ratio, by analyzing Fig. 5, shows that especially during the fed-batch assays 1 (FB1—fill time of 3 h), the increase in sulfate concentration (this is, from condition I to condition III) appeared to stimulate the complete oxidation of the substrate through sulfidogenesis, leading to removal of 89% of the fed substrate (Table 2),

which is the highest obtained efficiency. During the fed-batch assays 2 (FB2—6 h filling time), the decreasing COD/[SO_4^{2-}] ratio (this is, the increasing sulfate concentration) did not significantly improve sulfate removal. It can also be verified that compared to studies with reactors operating under similar conditions (substrate, biomass immobilization, cycle—[5]), but without supplementation of sulfate, a higher level of residual organic matter remained in the reactor, removing on average 77% of organic matter (Tables 2 and 3). The possible reasons for this lower organic matter removal can be attributed to incomplete oxidation of the substrate because of the short treatment cycle time (8 h) for the slower SRB metabolism and to a possible inhibition effect of sulfite on the biomass responsible for the organic matter removal.

It can be seen from Fig. 6 that a higher sulfate concentration in the influent (this is, from condition I to condition III for the respective feed strategies B, FB1 and FB2) increased the ratio between the removed volumetric sulfate load (RVSL) and the removed volumetric load of filtered organic matter ($RVOL_{SOF}$). This can be attributed to improved utilization of the organic matter for sulfate removal, or in other words, to growing favoritism of sulfidogenesis over methanogenesis. An exception was condition II-FB1, where the high organic matter removal (Fig. 2) was not followed by high sulfate removal (Fig. 3). This tendency of the increasing ratio between removed volumetric sulfate load (RVSL) and removed volumetric load of filtered organic matter ($RVOL_{SOF}$) was also noticed when fill time increased, i.e., from condition B to condition FB-2 at conditions I, II, and III of the COD/[SO_4^{2-}] ratio (Fig. 6). The exception again was condition II-FB1. Hence, increasing the fill time favored sulfidogenesis and disfavored methanogenesis.

The methane production fell significantly with increasing sulfate concentrations in the influent from condition I to condition III (i.e., less than 0.4±0.1 mmol CH_4 L^{-1} under condition I, less than 0.03±0.01 mmol CH_4 L^{-1} under condition II and no methane formation under condition III) for the respective feed strategies B, FB1, and FB2, indicating higher activity of the SRB during these conditions.

Comparing the sulfate removal efficiency to those from reactors used in other studies [1, 2], the reactor used in this work showed poorer performance, presenting lowest removal efficiency of 23% (at condition I-B) and highest of 71% (at condition I-FB2). However, it should be pointed out that the purpose of this study was not to optimize the sulfate removal during the implemented experimental conditions, but to evaluate the behavior of the reactor during the different feed strategies and different COD/[SO_4^{2-}] ratios, as well as to analyze the interaction effect. This objective has not yet been explored in the literature, which mainly deals with studies on sulfate removal analyzing the COD/[SO_4^{2-}] ratio in the influent, the type of substrate, and the reaction time, typically in continuous reactors.

Biomass Concentration and Microbiological Analyses

After the 302-day experimental stage, the solids adhered to the polyurethane foam were determined by removing the biomass with distilled water from approximately four cubes of the support. The washed foam cubes were then dried at 105°C for 24 h to determine the dry weight. The detached solids were quantified in accordance with the conventional methods presented in the APHA [8]. In this case, the foam presented a specific biomass concentration (C_{XTVS}' and C_{XTS}') of 1.3 g_{TVS}/g_{EPU} and 1.4 $g_{ST}.g_{EPU}^{-1}$, with approximately 82.1 g total volatile solids in the reactor (C_{XTVS}), which corresponds to 18.0 g TVS L^{-1} considering 4.0 L working volume in the reactor.

Sludge samples of the biomass immobilized on polyurethane foam, prior to reactor operation and after finishing the 302-day experimental stage, were assessed by means of

microbiological analyses using common optical and phase contrast microscopy. The main morphologies observed in the biomass prior to reactor operation were bacilli with rounded borders, probably acidogenic bacteria, as well as *Methanosaeta*-like cells. After the 302-day experimental stage the main morphologies observed were curved bacilli similar to sulfate-reducing bacteria, with minor amounts of cocus and bacilli with rounded borders. No *Methanosaeta*-like cells were detected. It can, therefore, be concluded that the experimental setup, during which the COD/[SO_4^{2-}] ratio was reduced by increasing the sulfate concentration and keeping the organic matter concentration constant (this is, from condition I to condition III), stimulated the development of curved bacilli similar to sulfate-reducing bacteria and impaired the development of *Methanosaeta*-like morphologies. The absence of morphologies similar to *Methanosaeta* in the sludge analyzed after reactor operation can be corroborated by the methane concentration profile under condition III (COD/[SO_4^{2-}] ratio of 0.34), which showed no methane in the biogas.

Conclusions

The best condition for sulfate removal was the 3-h fed-batch assay; however, on increasing the sulfate concentration in the influent stagnation in sulfate removal was observed. During the assays with a 6-h fill time, the removed sulfate load increased linearly, and in all conditions, the increase in sulfate concentration caused improved utilization of organic matter for the removal of sulfate, i.e., the ratio between removed sulfate load and removed organic matter load increased. However, in all operation modes, sulfate removal efficiency decreased when the applied sulfate load was increased, and total dissolved sulfide increased when the COD/[SO_4^{2}] ratio was reduced. Furthermore, methane production significantly decreased at higher sulfate concentrations, and the production of bicarbonate alkalinity remained stable and sufficiently high to guarantee absorption of the volatile acids and reactor stability.

With regard to the effect of fill time defined by the feed strategies, it can be concluded that increasing this variable favored the SRB in the competition for organic matter and disfavored other anaerobic microorganisms in the assimilation of substrate. Therefore, comparing the assays with the same COD/[SO_4^{2-}] ratios, sulfate removal was higher during the fed-batch assays than during the batch assays. Within this context, higher organic matter removal was observed during the batch assay with a COD/[SO_4^{2-}] ratio of 1.34. On increasing the fill time, this removal decreased. However, during the assays with COD/[SO_4^{2-}], ratios of 0.67 and 0.34 opposite behavior was seen, i.e., higher organic matter removal during the fed-batch assays in relation to the batch assays. With regard to the effect of the COD/[SO_4^{2-}] ratio, it can be concluded that during the batch and 6-h fed-batch assays, the removed load of filtered organic matter decreased as sulfate concentration in the influent increased, whereas during the 3-h fed-batch assays, increasing sulfate concentration improved organic matter removal. During the batch assays, the removed sulfate load increased significantly on reducing the COD/[SO_4^{2-}] ratio from 1.34 to 0.67 and remained at this level during the assay with a COD/[SO_4^{2-}] ratio of 0.34.

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