

Full-scale anaerobic sequencing batch biofilm reactor for sulfate-rich wastewater treatment

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ABSTRACT

This paper describes the performance and biofilm characteristics of a full-scale anaerobic sequencing batch biofilm reactor (ASBBR; 20 m³) containing biomass immobilized on an inert support (mineral coal) for the treatment of industrial wastewater containing a high sulfate concentration. The ASBBR reactor was operated during 110 cycles (48 h each) at sulfate loading rates ranging from 6.9 to 62.4 kgSO₄²⁻/cycle corresponding to sulfate concentrations of 0.58–5.2 gSO₄²⁻/L. Domestic sewage and ethanol were utilized as electron donors for sulfate reduction. After 71 cycles the mean sulfate removal efficiency was 99%, demonstrating a high potential for biological sulfate reduction. The biofilm formed in the reactor occurred in two different patterns, one at the beginning of the colonization and the other of a mature biofilm. These different colonization patterns are due to the low adhesion of the microorganisms on the inert support in the start-up period. The biofilm population is mainly made up of syntrophic consortia among sulfate-reducing bacteria and methanogenic archaea such as *Methanoseta* spp.

Keywords: Sulfate reduction; Anaerobic reactor; Mineral coal; Biofilm; Ethanol; Industrial wastewater

1. Introduction

High-sulfate wastewaters originate from a number of industrial activities, including pulp and paper manufacture, minerals processing, petrochemical industries and several industrial processes that use sulfuric acid as a raw material [1,2]. The biological removal of sulfate from wastewaters is a well-known process [3,4], but its application is still problematic due to the uncertainties regarding the stability and performance of full-scale reactors.

The conventional anaerobic sequencing batch reactor (ASBR) is operated under intermittent cycles or batches

of four stages: feeding, anaerobic biological reactions, biomass sedimentation and effluent discharge [5]. The sedimentation stage is directly dependent on the formation of biomass with good settling characteristics, such as granular sludge, avoiding losses of the metabolically adapted biomass during discharge of the treated wastewater.

The use of inert supports within a sequencing batch reactor is considered to be a promising method to improve the retention of solids and eliminate the uncertainty in biomass granulation. An ASBBR works with intermittent cycles of three stages: feeding, reaction and discharge, and it can operate at high cellular retention times by providing inert supports for cell adhesion and/or immo-

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bilization. The settling step after the reaction stage is not required as in conventional ASBR because the biomass is immobilized [6,7].

The anaerobic sequencing batch biofilm reactor (ASBBR) has been considered a potential alternative for sulfate-rich wastewater treatment. Sarti et al. [8] tested this bioreactor configuration at the pilot scale (1.2 m³) with mineral coal as the inert support over 185 days. The application of biological treatment to industrial effluent containing high sulfate concentrations (~200 gSO₄²⁻/L) provided significant sulfate reduction (88–92%) for influent concentrations of 0.25–3.0 gSO₄²⁻/L. Both domestic sewage and ethanol were added as sources of electrons for sulfate reduction.

Ethanol has previously been used as an electron donor in full-scale sulfate-reducing plants [4]. The main disadvantage of using ethanol as the electron donor is the generation of acetic acid, resulting in an effluent containing a high residual COD [9]. Consequently, the residual pollution caused by the electron donor should be minimized and sulfide should be partially re-oxidized to elemental sulfur in a separate second reactor [10,11].

This paper presents and discusses the behavior of a full-scale ASBBR for sulfate-rich wastewater treatment applied in an industrial plant. Domestic sewage was used as the primary electron donor and for diluting the industrial wastewater in order to obtain different sulfate concentrations (0.58–5.2 gSO₄²⁻/L). Mineral coal was used as the support material, and ethanol was also used as a supplementary electron donor for reasons of availability and cost. Studies must be conducted at a larger scale in order to evaluate the applicability of this anaerobic technology for industrial wastewaters.

2. Materials and methods

2.1. Sulfate-rich wastewater (industrial effluent)

The industrial sulfonation process used to transform vegetable oils (rice, soy, and corn) into sulfonated oils produces effluent wastewaters containing a high sulfate concentration. Sulfonation occurs in the presence of sulfuric acid (H₂SO₄) and liquid ammonia (25%) in a batch reactor operated under controlled temperatures. Free acids are eliminated from the reaction product by a washing operation that produces a highly toxic wastewater. The composition of the sulfate-rich washing wastewater is presented in Table 1.

2.2. ASBBR reactor characteristics and operational conditions

The full-scale ASBBR reactor was constructed from fiberglass with a total volume of 20 m³. The reactor was filled with 10,000 kg of irregular pieces of mineral coal (diameter 40–80 mm) occupying a volume of 18 m³, resulting in a liquid volume of 11 m³ (bed porosity = 0.39). The

Table 1
Characteristics of the industrial wastewater (20 samples)

Variables	Minimum	Maximum	Mean
pH	2.31	3.25	—
COD _{Total} g/L	9.24	15.43	13.7±4.1
COD _{Filtered} g/L	8.98	10.90	10.6±1.3
NH ₄ ⁺ g/L	1.32	1.87	1.52±0.5
SO ₄ ²⁻ g/L	183	284	201±35

utilization of a denser material (mineral coal) simplifies the reactor design by eliminating the need of an internal device to retain the bed particles.

The head-space volume (2.0 m³) was filled with 1.0 m³ of liquid to keep the recirculation pipe immersed in the liquid. Therefore, the treatment volume available in cycle or batch mode was 12 m³ (11 m³ + 1.0 m³). The outlet biogas pipe was immersed in a hydraulic seal containing an alkaline solution (NaOH) for H₂S removal.

The cycle time was 48 h, including the steps of feeding (1 h), reaction with continuous liquid recirculation (46 h) and discharge (1 h). The influent wastewater was pumped from a storage tank (15 m³) to a circular perforated tube located at the bottom of the reactor in order to achieve a better liquid distribution. Mixing was provided by liquid recirculation (up-flow) with a centrifugal pump (Munsch Aflon NP 40/200) connected to the inflow distribution system, with an initial recirculation flowrate of 10 m³/h (liquid velocity = 4 m/h). The discharge step was executed by another centrifugal pump (Jacuzzi 5A-T and flowrate = 6 m³/h), and the effluent was pumped for aeration to a tank in the wastewater treatment plant of the chemical industry Dissoltex (São Carlos/São Paulo-Brazil).

The reactor was maintained for 110 cycles (220 days) at an ambient temperature of 30±3°C. Domestic sewage was used to dilute the sulfate-rich industrial wastewater (Table 1), thus providing organic matter for sulfate reduction. Fig. 1 shows a schematic representation of the ASBBR reactor (full-scale) containing biomass immobilized on mineral coal, and Fig. 2 illustrates the installations of the ASBBR and the storage tank.

Table 2 summarizes the operational parameters applied to the ASBBR for two experimental periods. At the beginning (start-up) of the operation (period I; 71 cycles), the reactor was operated with a sulfate loading rate (SLR) of 18.7–62.4 kgSO₄²⁻/cycle (1.56–5.20 gSO₄²⁻/L). The SLR was decreased in the apparent steady-state period (period II; 29 cycles) to between 6.9 and 14.6 kgSO₄²⁻/cycle (0.58–1.23 gSO₄²⁻/L). Ethanol was added as a supplementary source of electrons for sulfate reduction. The added volume was varied according to the sulfate removal efficiencies obtained for the different COD/sulfate ratios (Table 2) with the aim of maximizing the simultaneous sulfate reduction and ethanol utilization.

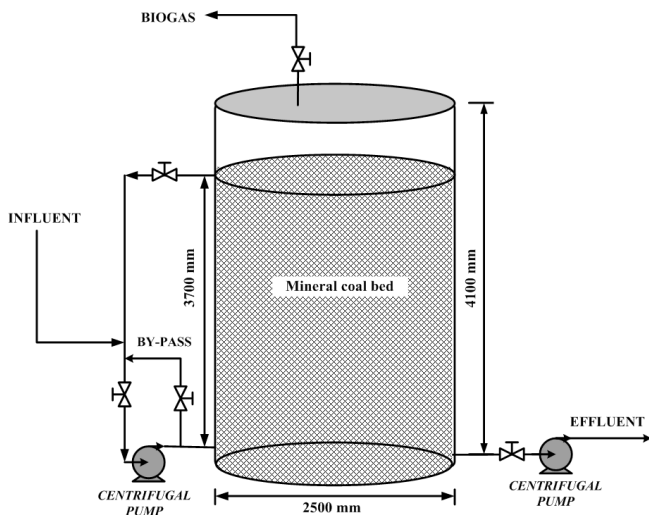


Fig. 1. Schematic representation of the ASBBR reactor (full-scale) containing biomass immobilized on mineral coal.



Fig. 2. Installation of the ASBBR (1) and the storage tank (2).

Table 2

Minimum-maximum results of the influent and effluent of an ASBBR reactor during start-up (period I) and steady-state (period II). OLR = organic loading rate (cycle); SLR = sulfate loading rate (cycle); ORR = organic removal rate (cycle); SRR = sulfate removal rate (cycle)

Parameters	Period I (influent)	Period I (effluent)	Period II (influent)	Period II (effluent)
Cycle numbers	1–71	1–71	72–110	72–110
^a Temperature, °C	25±3	29±4	30±3	32±3
pH	7.6–8.3	6.5–7.4	6.4–8.2	5.7–7.2
SLR, kgSO ₄ ²⁻ /cycle	18.7–62.4	—	6.9–14.6	—
SRR, kgSO ₄ ²⁻ /cycle	—	13.4–45.2	—	6.8–11.5
Sulfate, mg/L	1,560–5,200	440–1,435	575–1,230	2–26
^b TDS, mg/L	0.1–3.6	113–551	0.1–1.1	48–172
COD _{Total} /sulfate	1.5–2.4	—	3.7–5.7	—
OLR, kgCOD/cycle	39.2–119.7	—	25.7–66.5	—
ORR, kgCOD/cycle	—	13.5–56.3	—	10.2–19.1
COD _{Total} , mg/L	3,300–9,980	2,150–5,280	2,140–5,540	1,290–3,950
COD _{Filtered} ^c , mg/L	—	2,100–5,180	—	1,180–3,820
BA, mgCaCO ₃ /L	25–74	1,345–1,403	58–76	1,432–1495
VFA, mgHac/L	128–183	1,002–2,103	87–135	1,205–1,720
TSS, mg/L	137–208	50–175	189–258	76–134
VSS, mg/L	65–95	38–80	89–159	43–70

^aliquid (mean value) and ^btotal dissolved sulfide

The ASBBR was not inoculated with anaerobic sludge. Initially, the reactor was operated for 30 cycles (acclimatization phase; 60 days) to treat domestic sewage. After this phase, the industrial effluent with a high sulfate concentration was added to the influent. Domestic sewage was used to dilute the sulfate-rich industrial wastewater in the storage tank at different volumes depending on the desired influent sulfate concentration.

2.3. Reactor monitoring

Monitoring (92 cycles) was carried out through physical-chemical analyses of the influent and effluent samples, such as the chemical oxygen demand (COD) of total and filtered samples, total suspended solids (TSS), volatile suspended solids (VSS) and pH, according to the Standard Methods [12]. Determinations of volatile fatty

acids (VFA), such as acetic acid (HAC), and bicarbonate alkalinity (BA) followed the methodology described by Dilallo and Albertson [13] and modified by Ripley et al. [14]. The methylene blue method (method 4500 D) [12] was used to determine the total dissolved sulfide (TDS). Sulfate concentrations were measured by a turbidimetric method using the Hach SulfaVer® reagent. Influent and effluent samples were collected at the beginning and end of the same cycle, respectively.

The biomass concentration in the mineral coal was evaluated by analysis of the total volatile solids (TVS). Biomass concentration was assessed for each of the ten cycles, and estimated by drying the coal loaded-biomass at 105°C, and subsequently heating it in an oven at 540°C for 30 min to volatilize the biomass. The difference in the mineral coal weight before and after this process is reported as the biomass dry weight. The application of this methodology to mineral coal was used by Sarti et al. [8] and adapted from Nagpal et al. [9].

Microbial characterization using optical and UV fluorescence was also carried out to study the development of the microbial communities, their organization and the structure of the anaerobic biofilm. Optical and UV microscopic examinations were conducted using an Olympus BX60-FLA microscope equipped with system image analysis (Image-Pro Plus, version 4.1 for Windows).

3. Results and discussion

3.1. Performance of full-scale ASBBR reactor

The ASBBR reactor was monitored during 110 cycles for two operational periods characterized by different influent sulfate concentrations (Table 2). During start-up (period I; 71 cycles), the reactor achieved sulfate removal rates (SRR) of 13.4–45.2 kgSO₄²⁻/cycle for sulfate loading rates (SLR) of 18.7–62.4 kgSO₄²⁻/cycle (Table 2 and Fig. 3). In this period, the reactor presented a wide range of sulfate removal efficiencies (54–82%) (Table 2). This behaviour is related to the dilution of the sulfate-rich wastewater with sewage in the storage tank, which affects the influent sulfate concentration.

The mean sulfate concentrations of the influent and effluent were 3,070±1,048 mgSO₄²⁻/L and 948±262 mgSO₄²⁻/L, respectively (Fig. 2). The sulfate concentrations in the effluent remained between 440 and 1,435 mgSO₄²⁻/L and the COD/sulfate ratio at values of 1.5–2.4. As the COD/sulfate ratio was increased to improve sulfate reduction efficiency, higher consumption of ethanol occurred (1.8–2.9 kg ethanol/kgSO₄²⁻ removed/cycle).

As a stable condition was reached, sulfate removal efficiencies of 99% were achieved (Fig. 3) after the 71st day (period II; 29 cycles), when the SLR was reduced to the range of 6.9–14.6 kgSO₄²⁻/cycle (Table 2 and Fig. 3). The influent had mean sulfate concentration values of 808 ± 216 mgSO₄²⁻/L, and the effluent had 7.8 ± 6.5 mgSO₄²⁻.

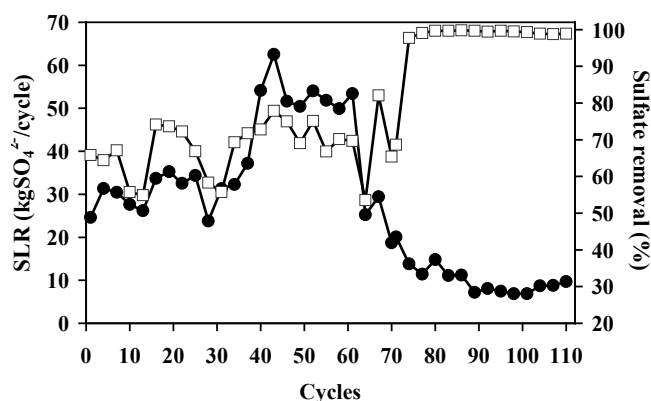


Fig. 3. SLR (sulfate loading rate) (●) and sulfate removal (□) during the experimental phase.

The higher efficiencies were attained by increasing the recirculation flow rate from 10 to 20 m³/h and with the COD/sulfate ratio ranging from 3.7 to 5.7 (Table 2). Sarti et al. [8] achieved similar results for sulfate removal with the same industrial wastewater in an ASBBR reactor using mineral coal as the inert support.

The increase of the COD/sulfate ratio in the apparent steady-state period occurred mainly by application of a lower concentration of sulfate (575–1,230 mgSO₄²⁻/L) and with the same added volume of ethanol at the end of start-up period (COD/sulfate = 2.0 and 2.1 kg ethanol/kgSO₄²⁻ removed/cycle). In fact, this strategy allows a greater availability of organic matter or electron donor for sulfate reduction [8], while the change in the recirculation flow rate (liquid velocity = 8 m/h) may reduce the influence of the liquid-phase mass transfer resistance in this type of reactor with a packed bed [15].

The liquid velocity can be manipulated to improve the reactor performance through the decrease of the liquid boundary layer that represents the resistance to mass transfer from the bulk liquid to the material support surface [16]. Ideally, the substrate, as sulfate and organic matter, must be transferred more easily from the liquid phase to the biofilm on the surface of the bioparticle (mineral coal).

After the acclimatization phase (30 cycles – 60 days), without seeding as described, the reactor achieved mean COD_{Total} removal efficiency of 43% in the start-up period (22–58%). In this period, the mean value of the influent COD_{Total} was 5,787 ± 1,956 mg/L (3,300–9,980 mg/L), with organic loading rates (OLR) ranging from 39.2 to 119.7 kg-COD_{Total}/cycle (Table 2 and Fig. 4). After 142 days (71 cycles) the mean effluent COD_{Total} and COD_{Filtered} values were 3,224 ± 898 mg/L and 3,142 ± 885 mg/L, respectively. The organic removal rates (ORR) were maintained between 13.5 and 56.3 kgCOD_{Total}/cycle.

The mean COD_{Total} removal efficiency was 45% in the steady-state period, similar to that of the previous

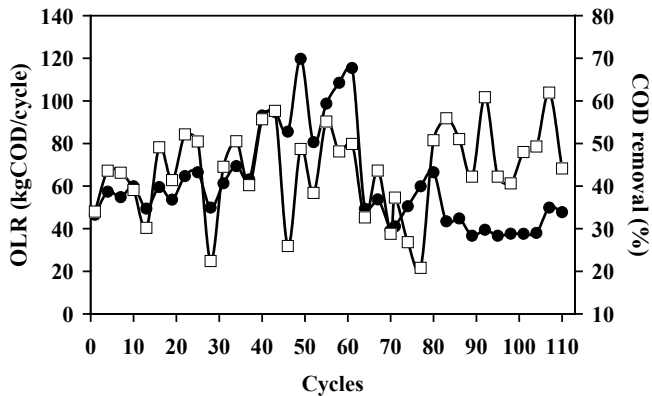
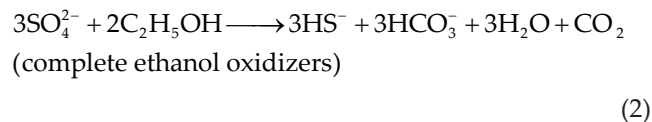
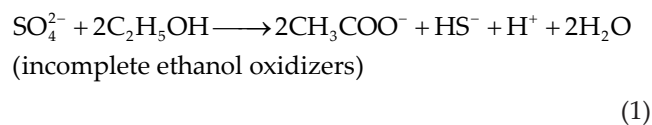


Fig. 4. OLR (organic loading rate) and (●) and COD removal (□) during the experimental phase.

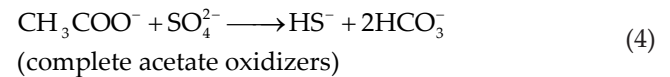
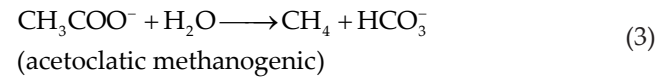
period. The reactor attained organic removal rates (ORR) of 10.2–19.1 kgCOD/cycle for organic loading rates (OLR) of 25.7–66.5 kgCOD_{Total}/cycle (Table 2 and Fig. 4). Effluent COD values remained between 1,290 and 3,950 mg/L as COD_{Total} and 1,180 and 3,820 mg/L as COD_{Filtered} while COD_{Total} removal efficiency values were 21–62%. The influent presented a mean COD_{Total} of 3,776 ± 787 mg/L (2,140–5,540 mg/L).

The main drawback of using ethanol as an electron donor for sulfate reduction is the generation of VFA as acetate, resulting in an effluent with significant residual COD. This high COD could be due to the strong competition among the sulfate-reducing bacterial species between incomplete ethanol oxidizers [Eq. (1)] and complete ethanol oxidizers [Eq. (2)] [9]. This fact can explain the low COD removal efficiencies and significant concentration of VFA in the effluent of the ASBBR reactor (Table 2). It is emphasized that the reactor effluent was pumped for treatment into the aeration tank of a wastewater treatment plant.



In period I (start-up) and period II (steady-state), the VFA values remained between 1,002 and 2,103 mgHac/L and 1,205 and 1,720 mgHac/L, respectively. This VFA was generated as a result of the partial oxidation of ethanol to acetate [Eq. (1)]. Therefore, because VFA was not totally consumed, the residual COD in the ASBBR effluent increased, resulting in the low COD removal efficiency obtained in the both operational periods (Fig. 4). On the other hand, the BA generation (Table 2) was considered

an indicator of VFA consumption by methanogenic microorganisms [Eq. (3)] and sulfate-reducing bacterial species as complete acetate oxidizers [Eq. (4)]. The BA values ranged from 1,345 to 1,403 mg CaCO₃/L (period I) and 1,432–1,495 mg CaCO₃/L (period II). The pH of the influent was 6.4–8.3, and the effluent pH was 6.5–7.4 (period I) and 5.7–7.2 (period II) (Table 2).



Concerning sulfide toxicity, it has been reported that the outcome of sulfide inhibition depends not only on the pH, which is directly related to the H₂S concentration, but also on the TDS concentration and the biomass characteristics. This indicates that both TDS and H₂S may inhibit microorganisms (sulfate-reducing bacteria and methanogenic microorganism) [17].

The effluent TDS concentrations obtained in this study are shown in Table 2. TDS mean concentrations decreased from 220 ± 108 mg/L (start-up period) to 95 ± 40 mg/L (steady-state period), with a maximum of 551 mg/L (minimum: 113 mg/L) and 172 mg/L (minimum: 48 mg/L), respectively. TDS values were reduced in the steady-state period, in which the sulfate concentrations were lower. Apparently, sulfide generation was not affected by the operation of the ASBBR reactor.

Table 2 presents the determination of suspended solids of ASBR reactors during the operational phase (110 cycles). The highest removal efficiencies of TSS and VSS during period II were 56% and 59%, respectively. The mean concentrations in the influent (110 cycles) were 193 ± 21 mg/L (TSS) and 98 ± 18 mg/L (VSS), with effluent TSS and VSS concentrations (period II) of 101 ± 16 mg/L (76–134 mg/L) and 52 ± 10 mg/L (43–70 mg/L), respectively. In period I, the suspended solids removal efficiencies were 49% (TSS) and 31% (VSS), with mean concentrations in the effluent of 89 ± 37 mg/L (50–175 mg/L) and 56 ± 12 mg/L (38–80 mg/L).

3.2. Mineral coal biofilm

The ASBBR influent, composed of domestic sewage, ethanol and sulfate-rich wastewater, allowed for the development of a diverse microbiota adhered to the inert support (mineral coal). The permanence and activities of the sulfate-reducing bacteria and methanogenic microorganism groups in biomass batch reactors depends, among other things, on the nature of the support component (for example, hydrophobicity) as well as on the adherence characteristics of the bacteria which form these communities [18,19].

Several studies have demonstrated the ability of sulfate-reducing bacteria to develop a biofilm under different conditions on various carriers [19–21]. In this case, the high sulfate reduction yields obtained in the ASBBR reactor, mainly in the steady-state period, indicate that sulfate-reducing bacteria were able to attach to the mineral coal, as previously found by Sarti et al. [8].

Operational stability (sulfate removal) was achieved after 71 cycles (steady-state period) following the changes in operating conditions, thus indicating the high capacity of the mineral coal to retain the biomass. Values of 0.145–0.261 kg STV/kg mineral coal were obtained in this period (Fig. 5). In the previous period, the biomass concentrations only varied from 0.112 to 0.145 kg STV/kg mineral coal. Fig. 5 shows that the difference between the values of biomass concentrations (0.033 kg STV/kg mineral coal) was small, so this behaviour is directly related to the low adhesion of the microorganisms on the inert support and the low suspended solids removal efficiency occurring in period I.

Examinations of biofilm samples under the microscope revealed the morphologies in the mineral coal (Fig. 6). Throughout the start-up and steady-state period, all observed microcolonies were mainly composed of non-fluorescent rods and filaments. These microorganisms are similar to sulfate-reducing bacteria (curved rod-shaped cells) (Figs. 6a and 6c) and *Archaea Methanosaeta*-like bacteria (filaments) (Figs. 6b and 6d).

Based on these observations and comparing them with the results obtained by Sarti et al. (2009) (in an anaerobic sequencing biofilm reactor treating sulfate-rich wastewater at 0.25–3.0 g $\text{SO}_4^{2-}/\text{L}$ and using mineral coal as an inert support), the biofilm population is mainly formed of syntrophic consortia among sulfate-reducing bacteria (*Desulfovibrio* spp.) and acetoclastic methanogenic bacteria (*Methanosaeta* spp.). Nevertheless, an increased sulfate-reducing bacterial population was detected in the steady-state period (Fig. 6c). These predominant morphologies, found by optical microscopy, are similar to *Desulfovibrio desulfuricans*. Sulfate removal efficiencies attained values of 99% in this period.

4. Conclusions

The main pathway for anaerobic biodegradation of sulfate-rich wastewater may vary between methanogenesis or sulfidogenesis according to several driving factors. However, in confined environments, final statements about potential competition between methanogenic and sulfate-reducing microorganisms should be associated first with each particular bioreactor's configuration, feeding influent characteristics and microbial composition.

Comparing the responses of the full-scale ASBBR reactor and biomass composition, it can be concluded that this reactor configuration has sulfate removal as the main target, despite the changes observed in the biomass

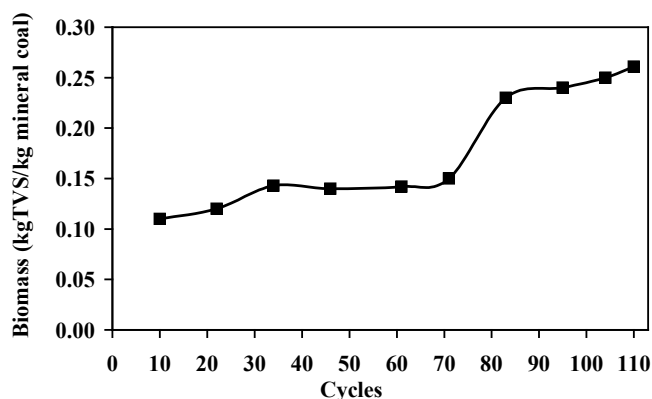


Fig. 5. Biomass concentration in the mineral coal in the ASBBR operation.

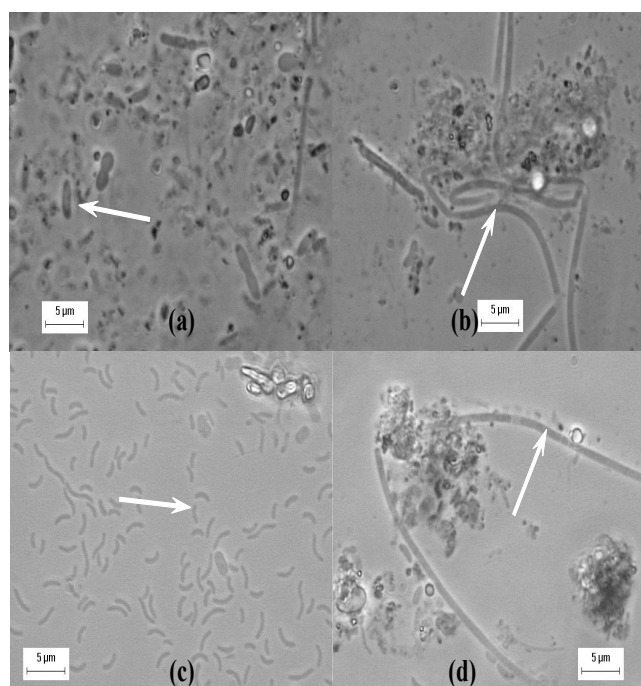


Fig. 6. Morphologies attached to mineral coal sampled during the start-up period (a and b) and the steady-state period (c and d). Biofilm composition with some curved rod-shaped cells similar to sulfate-reducers (a) and *Methanosaeta*-like cells (filaments) and rods (b). Biofilm population showing mainly curved rod-shaped cells similar to *Desulfovibrio* spp. (c) and *Methanosaeta* spp. filaments) (d).

characteristics and consequent variations in the metabolic interactions due to the different operating conditions applied throughout the experiment. The results can be interpreted by considering the syntrophic relationships among acidogenic bacteria, acetoclastic methanogenic archaea and sulfate-reducing bacteria. It was observed that mineral coal is an effective inert support for biomass

attachment, especially for methanogenic archaea (*Methanosaeta* spp.) and sulfate-reducing bacteria (*Desulfovibrio* spp.).

The ASBBR reactor was monitored during 110 cycles (48 h/cycle) at sulfate loading rates ranging from 6.9 to 62.4 kgSO₄²⁻/cycle, corresponding to sulfate concentrations of 0.58–5.2 gSO₄²⁻/L. High sulfate removal efficiencies (99%) were attained in the steady-state period of operation; however, the existence of reduced sulfur compounds (TDS) and residual COD was observed. The residual COD is related to the use of ethanol for biological sulfate reduction. Biological inhibition was not observed with the sulfide production problems in the operation of the reactor.

The application of this process in a full-scale ASBBR reactor would require a post-treatment system to adequately treat the effluents produced to meet emission standards. The residual COD composed of organic acids (for example, acetic acid) can be easily removed in biological reactors (aerobic and anaerobic).

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Abbreviations

ASBBR	— Anaerobic sequencing batch biofilm reactor
BA	— Bicarbonate alkalinity, mgCaCO ₃ /L
COD	— Chemical oxygen demand
FSBR	— Falling sludge bed reactor
OLR	— Organic loading rate
ORR	— Organic removal rate
SLR	— Sulfate loading rate
SRR	— Sulfate removal rate
STV	— Total dissolved sulfide, mg/L
TDS	— Total dissolved sulfide, mg/L
TSS	— Total suspended solids, mg/L
UASB	— Upflow anaerobic sludge bed
VFA	— Volatile fatty acids concentration, mg HAC/L
VSS	— Volatile suspended solids, mg/L

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