Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 32, No. 01, pp. 59 - 71, January - March, 2015
dx.doi.org/10.1590/0104-6632.20150321s00003158

COMPARISON OF UASB AND FLUIDIZED-BED REACTORS FOR SULFATE REDUCTION

S. M. Bertolino, L. A. M. Silva, S. F. Aquino and V. A. Leão*

Federal University of Ouro Preto, Bio&Hydrometallurgy Laboratory, Department of Metallurgical and Materials Engineering, Campus Morro do Cruzeiro, s.n., Bauxita, 35400-000, Ouro Preto - MG, Brazil.
Phone: + (55) (31) 35591102, Fax: + (55) (31) 35591561
E-mail: versiane@demet.em.ufop.br; versiane.ufop@gmail.com

(Submitted: December 8, 2013 ; Revised: May 1, 2014 ; Accepted: May 6, 2014)

Abstract - Reactor hydrodynamics is important for sulfidogenesis because sulfate reduction bacteria (SRB) do not granulate easily. In this work, the sulfate reduction performance of two continuous anaerobic bioreactors was investigated: (i) an upflow anaerobic sludge blanket (UASB) reactor and (ii) a fluidized bed reactor (FBR). Organic loading, sulfate reduction, and COD removal were the main parameters monitored during lactate and glycerol degradation. The UASB reactor with biomass recirculation showed a specific sulfate reduction rate of 0.089±0.014 $g.gSSV^{-1}.d^{-1}$ (89% reduction), whereas values twice as high were achieved in the FBR treating either lactate (0.200±0.017 $g.gSSV^{-1}.d^{-1}$) or glycerol (0.178±0.010 $g.gSSV^{-1}.d^{-1}$). Sulfate reduction with pure glycerol produced a smaller residual COD (1700 $mg.L^{-1}$) than that produced with lactate (2500 $mg.L^{-1}$) at the same $COD.sulfate^{-1}$ mass ratio. It was estimated that 50% of glycerol degradation was due to sulfate reduction and 50% to fermentation, which was supported by the presence of butyrate in the FBR effluent. The UASB reactor was unable to produce effluents with sulfate concentrations below 250 $mg.L^{-1}$ due to poor mixing conditions, whereas the FBR consistently ensured residual sulfate concentrations below such a value.

Keywords: Sulfate reduction; Anaerobic processes; Fluidized bed bioreactors; Glycerol; Upflow anaerobic sludge blanket; Wastewater treatment.

INTRODUCTION

Treatment of sulfate-containing effluents is a major issue for both mining, metallurgical, and chemical industries due to a frequently large anion content (INAP, 2003). The reasons for such contamination are the widespread use of sulfuric acid in chemical and metallurgical industries, in addition to the natural oxidation of sulfide minerals in mining operations.

Sulfate is not a very toxic compound, but above 600 $mg.L^{-1}$ in drinking water, it usually has laxative effects. Therefore, the World Health Organization (WHO) does not establish a guideline value for sulfate and only recommends that authorities should be notified when the anion concentration is above 500 $mg.L^{-1}$ in drinking water. Conversely, since the presence of sulfate in concentrations higher than 250 $mg.L^{-1}$ may affect the acceptability of drinking water, this concentration is usually taken as a target from a water quality perspective. Regarding wastewater, most countries do not specify a value for sulfate, but limits on maximum total dissolved solids (TDS) are usually set, implying that sulfate concentrations must comply with such limits (INAP, 2003). Overall, discharge limits varying between 250 $mg.L^{-1}$ and 500 $mg.L^{-1}$ are commonplace in mining countries, requiring effluent treatment when sulfate concentrations are above such threshold values (WHO, 2011).

Among the high-rate anaerobic reactors applied to sulfate reduction, the UASB reactor and the FBR

*To whom correspondence should be addressed

are the most studied. Ideally, both reactors must ensure a high concentration of active biomass along with good mixing conditions so that high performances can be achieved (Kato *et al*., 1994; Nielsen, 1987; Omil *et al*., 1996). Furthermore, in the case of UASB reactors, the residence time must be larger than the generation time to avoid microorganism washout (during sulfidogenesis) (Kaksonen *et al*., 2004). Overall, the performance of anaerobic reactors treating high sulfate loading rates (SLR) is defined by: (i) substrate type (Liamleam and Annachhatre, 2007); (ii) COD.sulfate^{-1} ratio (Shayegan *et al*., 2005; Velasco *et al*., 2008); (iii) inoculum source and enrichment procedure (Mohan, 2005); (iv) pH values (Cao *et al*., 2009); (v) competition among different groups of microorganisms (Dar *et al*., 2008; Zhao *et al*., 2008), and reactor configuration (Sahinkaya *et al*., 2007; Sheoran *et al*., 2010). Moreover, competition between sulfate-reducing bacteria (SRB) and methane-producing microorganisms (MPM) in anaerobic reactors is well documented (Bhattacharya *et al*., 1996; Harada *et al*., 1994; Omil *et al*., 1998), but the fermentative metabolism, which can also degrade low molecular weight carbon sources (Dinkel *et al*., 2010; Ren *et al*., 2007; Zhao *et al*., 2008), is less discussed in the context of continuous sulfate reduction.

It is also worth emphasizing that the main barriers for the widespread implementation of a biological alternative for sulfate removal are both the cost of organic matter and the need for downstream COD removal. An alternative organic substrate could be crude glycerol (g-phase). This is a by-product of biodiesel production that contains approximately 50–60% glycerol, 12–16% alkali soaps and hydroxides, 15–18% methyl-ethers, 8–12% methanol and 2–3% water. With the development of the biodiesel industry, a surplus of crude glycerol is foreseen, but it has been tested mostly as a substrate for methane production (Álvarez *et al*., 2010; Fountoulakis and Manios, 2009; Lopez *et al*., 2009; Yang *et al*., 2008), and only a few studies have addressed glycerol application as a potentially inexpensive carbon and electron source for SRB growth (Dinkel *et al*., 2010; Qatibi, 1990). Therefore, this work initially sought to assess the performance of two different bioreactors treating sulfate-laden waters: (i) an UASB reactor, which has a simple and inexpensive design and does not require a supporting material for bacterial growth, and (ii) a FB reactor, in which activated carbon was utilized as support. The second goal was to investigate the use of pure glycerol as a carbon source for sulfate reduction in the reactor with the best performance (fluidized bed reactor), as a preliminary step before investigating the use of crude glycerol.

MATERIALS AND METHODS

Bioreactors

Two lab-scale bioreactors were projected and assembled as shown in Figure 1. Both reactors were placed inside a fume hood in a temperature-controlled room, whereby the temperature was maintained at 25±2 ºC (simulating an industrial operation). Peristaltic pumps fed a Postgate C medium supplemented with sulfate (0.5 g.L^{-1} KH_2PO_4; 1.0 g.L^{-1} NH_4Cl; 0.06 g.L^{-1} $MgSO_4.7H_2O$; 0.1 g.L^{-1} $FeSO_4.7H_2O$; 0.25 g.L^{-1} yeast extract; 2.96 g.L^{-1} Na_2SO_4; and 3.76 g.L^{-1} lactate as carbon and electron source) into both reactors. The UASB reactor has been previously described (Bertolino *et al*., 2012). The operational conditions followed in this paper are detailed in Table 1.

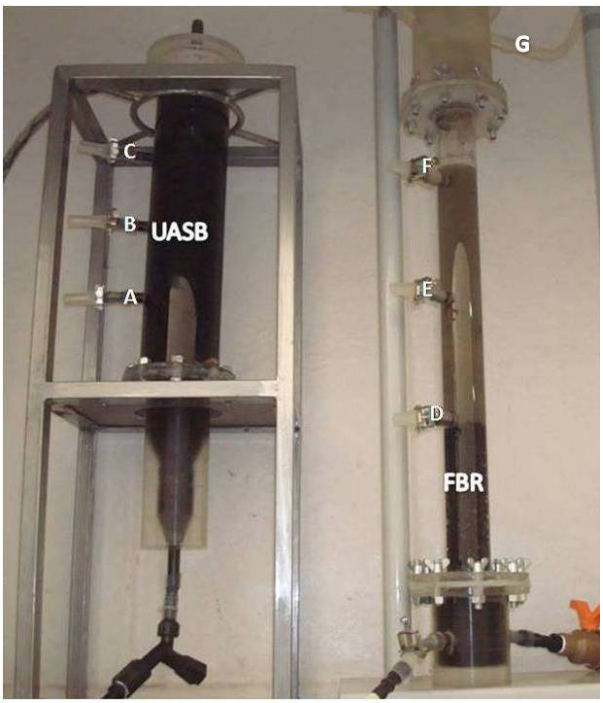

Figure 1: Pictures of the two lab-scale reactors, UASB and FBR. The UASB reactor had a total volume of 3.0 L and contained three sampling ports (*a*, *b* and *c*). Port *c* was utilized for biomass recirculation during phase VII. In the FBR biomass recirculation was performed from point *g*. The total volume of the FBR was 1.3 liter. Three sampling ports (*d, e* and *f*), a gas outlet (*g*), a feed tank.

Table 1: Characteristics and operating conditions of the reactors studied.

Parameters	UASB	FBR
Volume (L)	3.0	1.3
Flow rate ($L.h^{-1}$)	0.125-0.167±0.01	0.13±0.01
Upflow velocity ($m.h^{-1}$)	0.125 (phase I-V) 0.167 (phase VI) 1.75 (phase VII)	75
Hydraulic retention time (h)	24±1 (phases I-V; VII) 18 ±1 (phase VI)	10±1
Recirculation rate ($L.h^{-1}$) Temperature Carrier material Fluidization (%)	12 25±2 - -	166 25±2 activated carbon 86

The total volume of the FBR was 1.3 liters. Three sampling ports (D, E, and F), a gas outlet (G), a feed tank, and an effluent tank completed the system. Activated granulated carbon *(Synth)* was used as the biomass carrier material (150 g; 2.1 mm mean diameter; density: 1.63 $g.cm^{-3}$), and it was fluidized by means of flow recirculation by a second pump with the flow rate set at 166 $L.h^{-1}$. This resulted in an upflow velocity of 75.0 $m.h^{-1}$ and 86% of bed expansion (Table 1). For fluidization, the effluent from the outlet port (G) was recycled into the system.

Microorganisms and Reactor Start-Up

The original inoculum (granular sludge) was obtained from a UASB reactor (real scale) treating domestic wastewater. Enrichment of sulfate-reducing bacteria was performed in a batch reactor (5 liters) with a Postgate C mineral medium (as described in the previous section).

The time diagram depicted in Figure 2 shows the experimental conditions applied in each reactor. The Postgate C medium, with variable sulfate and lactate concentrations (Table 2), was applied for growth. During the FBR operation, the sulfate concentration was kept at 2.0 $gSO_4^{2-}.L^{-1}$ in phases I and II, while in phases III and IV, the COD was set at 5.0 $g.L^{-1}$. The optimum COD.$sulfate^{-1}$ ratio (2.5) was applied during phase V, aiming at preparing the FBR for a substrate change (from lactate to glycerol). Phase VI was run with glycerol as the only carbon source, since it replaced lactate in the Postgate C medium. Similarly, the operational conditions for the UASB reactor were as follows: reactor start-up during phases I and II; COD increasing from 3.6 $gCOD.L^{-1}$ to 6.0 $gCOD.^{-1}$ in phases III to V; flow rate change from 0.125 $L.h^{-1}$ to 0.167 $L.h^{-1}$ (HRT reduced from 24h to 18h) (phase VI); and effluent recirculation during phase VII (Bertolino *et al*., 2012).

The effects of COD.$sulfate^{-1}$ mass ratio, upflow velocities (UASB), and substrate type (FBR) in the performance of both reactors were assessed. To accomplish this, the reactor effluents were analyzed twice a week for total filtered chemical oxygen demand (COD), sulfate, alkalinity, volatile fatty acids (VFA), volatile suspended solids (VSS), pH, and redox potential (Eh). Once a week, a sample from inside the reactor was withdrawn for measuring VSS, alkalinity, pH, and redox potential, whereas viable cells were determined monthly.

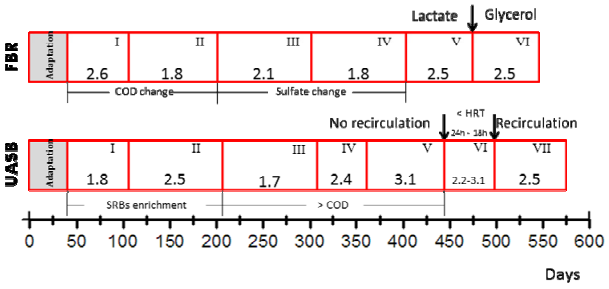

Figure 2: Time diagram showing experimental conditions applied in both the UASB reactor and the FBR. Inside each box is depicted the COD.$sulfate^{-1}$ mass ratio. When there was a change on the COD or sulfate loading the other parameter was kept constant. During phase VI (UASB reactor), the change on the COD.$Sulfate^{-1}$ ratio was due to different flow rate applied.

Table 2: COD and sulfate concentrations applied during the operation of both FBR and UASB reactors (mean values ± standard deviation).

Parameter	Reactor type	Phases monitored						
		I	II	III	IV	V	VI	VII
COD (mgL^{-1})	FBR	5086±276	3690±266	5026±250	5007±303	5122±242	4916±503	-
	UASB	3546±330	5007±284	3624±254	4743±300	6040±411	5226±800	5200±332
Sulfate (mgL^{-1})	FBR	1989±082	2096±082	2385±137	2756±138	2068±135	1990±082	-
	UASB	1967±189	1964±101	2122±124	2017±188	1944±97	1966±161	2069±140

Analytical Methods

Sulfate concentration was determined by ionic chromatography (Metrohm) using an ASSUP-10 column and conductivity detection. Prior to the analysis, cupric chloride was added to the reactor effluent sample to precipitate sulfide. The pulp was then filtered (0.22 μm membrane filters), and the aqueous phase was analyzed. VFA (acetic, propionic, valeric, and butyric) were determined by high-performance liquid chromatography, (HPLC, Shimadzu), with an ion exchange column Aminex HPX-87H 300mm x 7.8mm (Bio-Rad). Prior to injection, samples were filtered using 0.22 μm membrane filters (Millipore). Bicarbonate alkalinity (BA), VSS, and COD analysis were carried out according to the Standard Methods for Water and Wastewater (APHA, 2012). Before COD determination, any sulfide present in effluent samples was stripped off by adding a drop of HCl (35%) and flushing the sample for 10 min with N_2. The solution's pH (Hanna HI931400) and its redox potential (Digimed) (vs. an Ag-AgCl electrode) were also recorded.

Microorganisms in the liquid phase (free cells) were quantized by a 3-tube most probable number (MPN) procedure utilizing the Postgate C medium for SRB growth. Prior to the experiments, culture tubes were degassed with pure N_2, sealed, and autoclaved (120 °C, 1.5 atm, 20 min). Subsequently, culture tubes plus the control were incubated for 30 days at 35 °C.

Bacterial Diversity

Gene sequences were utilized to determine the bacterial phylogeny and taxonomy present in the sludge of both reactors. Genomic DNA from a mixed culture of SBR representing the different operational conditions of both reactors was extracted and purified using the CTAB/NaCl 10% method. The quality of the DNA was analyzed on a 0.6% agarose gel (w/v). For PCR amplifications, the initial DNA concentration was determined by spectrophotometry at 260 nm (SHIMADZU UV—1601 spectrophotometer) and adjusted to 50ng/L. Subsequently, PCR amplification cloning and sequencing of both 16S-23S rRNA Intergenic and *dsr*B (for SRB), as well as 16S rRNA (for fermentative) gene fragments, were carried out. All samples were cloned into the pGEMT-Easy vector and then sequenced in an ABI 3100 automated sequencer (Applied Biosystems) using a dye terminator kit. The sequences were then used for phylogenic analysis. The experimental procedures were fully described in Rampinelli *et al*. (2008) and Rodrigues (2012).

RESULTS

The performance of the UASB reactor treating lactate was previously discussed (Bertolino *et al*., 2012). It is utilized in the present paper as a reference for analyzing the performance of the FB reactor.

Reactor Performance

Although both reactors were started up with the same inocula, which were enriched with the same growth medium (Postgate C), their performances were quite distinct. The higher residence time in the UASB reactor (24 hours) would imply better performance as compared to the FBR (10-hour residence time). However, such an outcome was not observed due to the different reactor configurations. A high sulfate reduction efficiency (>90%) was observed in the FBR as soon as the adaptation phase ended (Figure 3A), resulting in residual sulfate concentrations below 250 $mg.L^{-1}$ already in phase I. Similar behavior was not observed in the UASB reactor, which showed sulfate removal efficiencies between 36% and 66% (Figure 3B) during the phases in which the reactor operated without biomass recirculation (I-VI). Nevertheless, when the upflow velocity changed from 0.024 $m.h^{-1}$ to 1.75 $m.h^{-1}$ (phase VII), sulfate removal increased to 89%.

Worldwide discharge limits for sulfate in industrial wastewaters vary between 250 $mg.L^{-1}$ and 500 $mg.L^{-1}$ (INAP, 2003; WHO, 2011). For a target value of 250 $mg.L^{-1}$, it can be seen in Figure 3A that, during phases I, III, V, and VI, the FBR consistently produced residual sulfate concentrations below that limit. In contrast, the UASB reactor was unable to produce final sulfate concentrations lower than 250 $mg.L^{-1}$, with average outlet sulfate concentrations around 800 $mg.L^{-1}$ (Figure 3B) during phases I to VI (Bertolino et al., 2012). Recirculation improved sulfate reduction during phase VII, but the final sulfate concentration was still 275 ± 106 $mg.L^{-1}$ (Table 3).

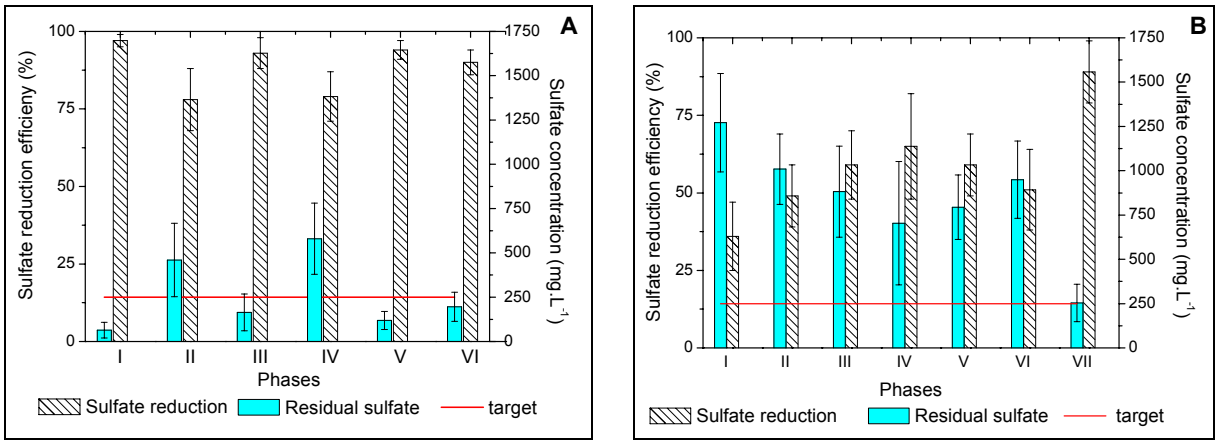

Figure 3: Sulfate reduction, residual and target sulfate concentrations in different phases of FBR (A) and UASB reactor (B) operations.

Table 3: Best parameters achieved during sulfate reduction in the UASB reactor and the FBR, treating synthetic sulfate wastewater with lactate (phases VII - UASB reactor and I – FBR) or glycerol (phase VI - FBR).

Parameters	Unit	UASB		FBR	
		III	VII	I	VI
Chemical oxygen demand (COD)	$mg.L^{-1}$	3624±254	5200±320	5086±276	4916±503
Organic loading rate (OLR)	$gCOD.L^{-1}.d^{-1}$	3.25±0.25	5.04±0.33	12.34±0.98	11.54±1.19
Sulfate loading rate (SLR)	$gSO_4^{2-}.L^{-1}.d^{-1}$	2.08±0.13	2.0±0.14	4.82±0.32	4.67±0.20
Residual sulfate conc.	$mg.L^{-1}$	882±257	275±106	78±10	90±4
pH	-	7.7±0.3	7.7±0.3	8.4±0.1	7.5±0.2
Volumetric COD removal rate	$gCOD.L^{-1}.d^{-1}$	1.44±0.45	1.94±0.56	6.25±0.63	7.44±1.68
Volumetric sulfate reduction rate	$gSO_4^{2-}.L^{-1}.d^{-1}$	1.22±0.24	1.60±0.26	4.67±0.35	4.21±0.25
Sulfate reduction efficiency	%	59±11	80±8	97±2	90±4
COD removal efficiency	%	40±11	39±11	51±5	64±12
Overall biomass concentration	$gVSS.L^{-1}$	16.0	18.0	25.6	25.3
Mean specific sulfate reduction rate	$gSO_4^{2-}.gVSS^{-1}.d^{-1}$	0.077±0.12	0.089±0.014	0.200±0.017	0.178±0.010
Mean specific COD removal rate	$gCOD.gVSS^{-1}.d^{-1}$	0.09±0.028	0.108±0.031	0.266±0.027	0.314±0.071

The alkalinity profile (Figures 4A and 4B) was also an important parameter in the assessment of the sulfate reduction performance. This was because the alkalinity is a product of incomplete substrate oxidation (of either lactate or glycerol) by different SRB groups (*Desulfovibrio, Desulfobulbus*, *Desulfotomaculum*, *and Desulfomona*). Another important aspect to be assessed was related to the volatile fatty acid (VFA) concentration. VFAs were produced due to lactate/glycerol fermentation, causing a pH decrease in the reactor. Therefore, the VFA–alkalinity balance ultimately defined the reactor pH profile, as shown in Figures 4C and 4D. The larger alkalinity production in both reactors (Figures 4A and 4B) enabled the pH values (Figure 4C and 4D) to be maintained in the optimum range for SRB growth (Barton, 1995), without any external alkalinity requirement. Thus, in the FBR, pH values varied between 7.9 and 8.8, with a mean value of 8.4 (Figure 4C) when lactate was the carbon source, dropping to 7.5 when glycerol was the substrate.

As sulfate reduction (and alkalinity production) was high in the FBR, a quite stable operation was observed. Going from phase I ($COD.sulfate^{-1}$ mass ratio of 2.6) to phase 2 ($COD.sulfate^{-1}$ mass ratio of 1.8), there was a reduction in both alkalinity and VFA concentrations (Figure 4A), which is likely a consequence of decreasing both organic loading rates (OLR) (Figure 5A) and sulfate reduction rates (Figure 5B) in the reactor. The FBR showed a tendency toward stabilization during the remaining phases of treating lactate (III to V), which was reflected in the values of the free SRB population above 10^9 free $cells.mL^{-1}$ (Figure 6A). The large data scattering observed in the VFA figures during phase IV (Figure 4A) can be ascribed to an increase in the sulfate loading rate (SLR), from 5.61±0.29 $gSO_4^{2-}.L^{-1}.d^{-1}$ in phases I and II to 6.46±0.34 $gSO_4^{2-}.L^{-1}.d^{-1}$ (Figure 5B) in the FBR.

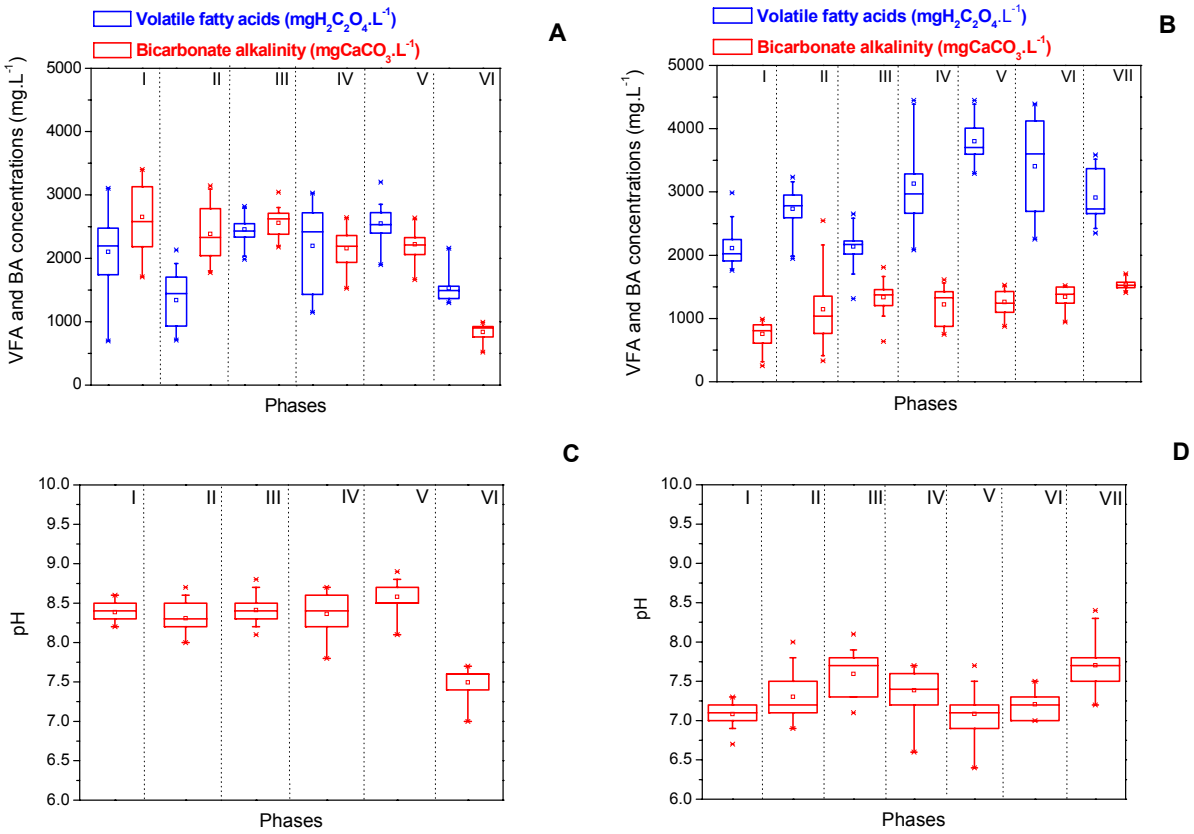

Figure 4: Performance parameters in different phases (according to the time diagram, Figure 2) in the FBR (A and C) and the UASB reactor (B and D). VFA: volatile fatty acids; BA: bicarbonate alkalinity.

That impaired the sulfate removal efficiency, which was reduced from 93% (phase III) to 79% during phase IV (Figure 3A). In the best operational conditions (phases III and V), a VFA.alkalinity^{-1} ratio around 1 was observed. Both VFA and alkalinity were reduced during phase VI, as will be discussed later.

In the UASB reactor during the phases without recirculation (I to VI), alkalinity production increased in phases I and 2 and stabilized in the 1300–1500 mg/L range during phase III, as show in Figure 4B, whereas pH values increased from 7.1 (phase I) to 7.6 (phase III), as shown in Figure 4D. During phases IV and V, the reduction in pH values (7.6 to 6.9, Figure 4D) were ascribed to increased OLR (from 4.65±0.30 g.L^{-1}.d^{-1} to 5.89±0.48 g.L^{-1}.d^{-1}) (Figure 5C), which implied larger VFA production (Figure 4C). Biomass recirculation (phase VII) enabled stabilization of both VFA and alkalinity, which resulted in higher pH values (7.5, Figure 4D) and can be related to the recovery and stabilization of the SRB population, which increased from 7x10^{8} in phase VI to 10^{10} free cells/mL in phase VII (Figure 6B).

The COD consumption rate for a 10-hour residence time in the FBR varied from 4.05±0.85 gCOD.L^{-1}.d^{-1} (minimum removal efficiencies: 47±9%) in phase II to 7.08±1.34 gCOD.L^{-1}.d^{-1} (maximum removal efficiency: 59±10%) in phase V (Figure 5A). Conversely, in the UASB reactor, despite a longer residence time (24 hours), lower removal rates were observed, from 0.88±0.52 gCOD.L^{-1}.d^{-1} (13±7% removal) in phase VI to 1.50±0.52 gCOD.L^{-1}.d^{-1} (41 ± 11% removal in phase IV) in those phases where no recirculation was applied. Mixing conditions might have accounted for such behavior because, when the recirculation was performed in the UASB reactor (phase VII), COD consumption (Table 3) increased 30% to 1.94±0.56 gCOD.L^{-1}.d^{-1}), which was still lower than that observed in the FBR. In addition, data scatter was more pronounced in the UASB reactor as compared to the FBR, confirming the lower operational stability of the former.

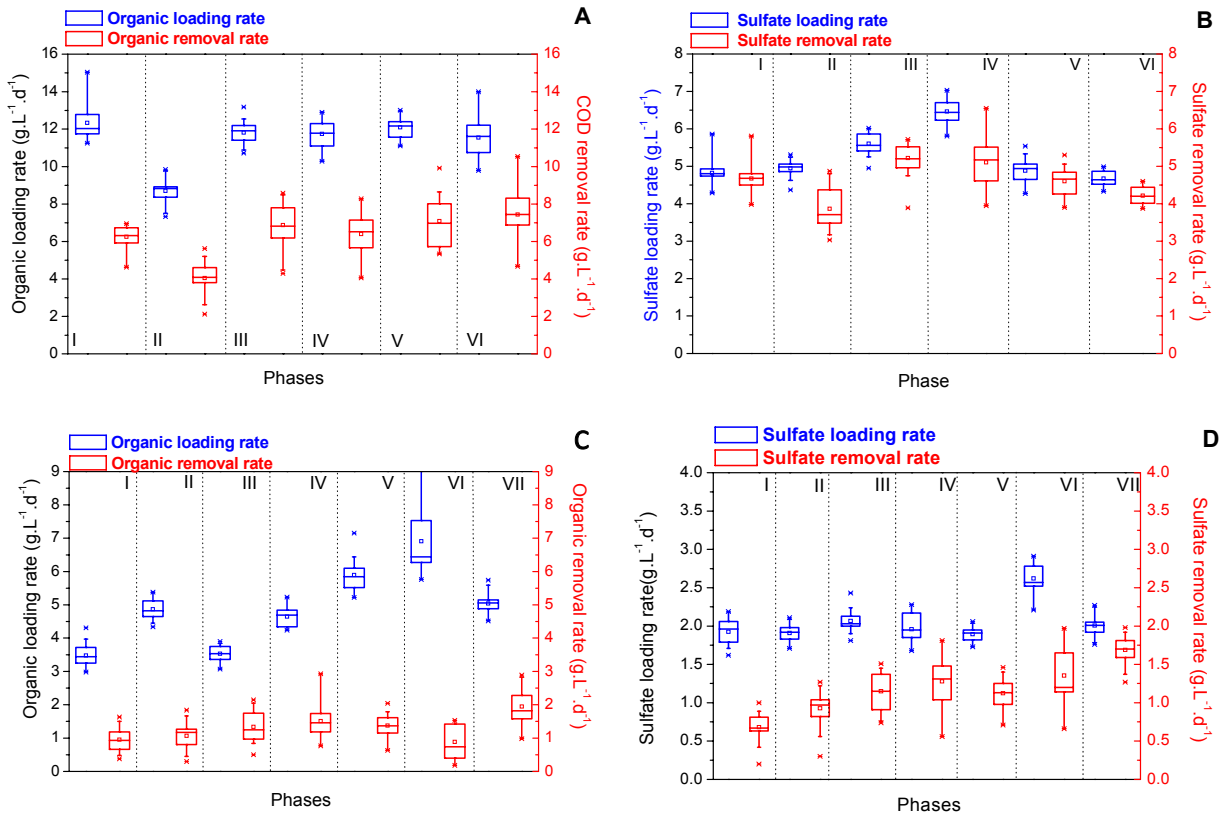

Figure 5: Volumetric organic and sulfate loading rates applied in the UASB reactor and the FBR. Organic loading and COD removal rates in the FBR (A) and UASB reactor (C); sulfate loading and removal rates in the FBR (B) and UASB reactor (D).

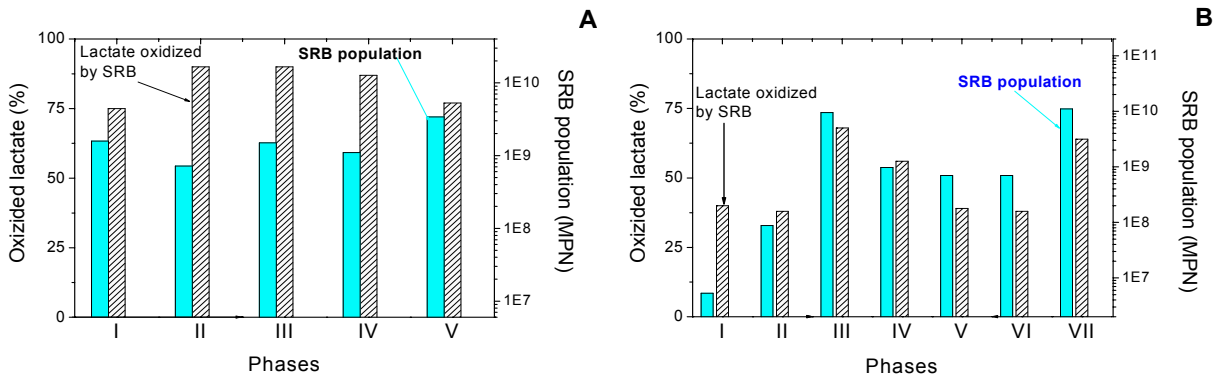

Figure 6: SRB population and lactate oxidized by SRB during continuous sulfate removal in the FBR (A) and UASB reactor (B). Glycerol was utilized as substrate in phase VI during the operation of the FBR and therefore does not appear in Figure A.

Effect of the Reactor Configuration on Sulfate Removal

Mass transfer effects play an important role in the performance of high-rate anaerobic reactors (Kato *et al.*, 1994), and this was particularly important for sulfidogenesis in the UASB reactor. How the biomass grew and was maintained in both reactors affected the enrichment step and therefore the competition with fermentative bacteria.

In a standard UASB reactor, mixing is provided by both the upflow velocity and gas bubbles (produced

by methanogens), which maintain the suspended sludge. Several studies have shown that granular sludge formation is related mainly to the presence of methanogens (Liu *et al*., 2003; Schmidt and Ahring, 1996). However, methanogens are highly sensitive to high sulfide concentrations, whereas propionibacteria, which are nucleation centers of the granules, are outcompeted by SRB in the presence of sulfide (Oyekola *et al*., 2009). Therefore, in the current work, an important limitation of the UASB reactor in treating sulfate was the granulation of the biomass (Speece, 1983). Indeed, the fine and weightless granular sludge observed in the UASB reactor was prone to washout. Such a phenomenon occurred during phase VI, when the flow rate was increased from 0.125 $L.h^{-1}$ (residence time of 24 h) to 0.167 $L.h^{-1}$ (residence time of 18h) and the upflow velocity was increased from 0.018 $m.h^{-1}$ to 0.024 $m.h^{-1}$ (Table 1) in an attempt to improve the mixing conditions in the vessel. This resulted in increased VSS concentrations in the UASB effluent, from 80 $mg.L^{-1}$ (on average) to nearly 500 $mgSSV.L^{-1}$ toward the end of the phase. Such biomass loss impaired the reactor performance with a drop in both COD consumption (from 1.6 $g.L^{-1}.d^{-1}$ in phase V to 0.8 $g.L^{-1}.d^{-1}$ in phase VI) (Figure 5C) and sulfate removal efficiencies (which progressively decreased from 70% to 40% during phase VI, as depicted in Figure 5D). Omil *et al*. (1996) also reported that increasing the upward velocity can impair sulfidogenesis.

Because increasing the superficial upflow velocity resulted in bacterial washout, biomass recirculation was tested so that the upward velocity was increased to 1.75 $m.h^{-1}$, thus improving mass transfer in the UASB reactor. Accordingly, sulfate reduction was improved to 89% (specific activity of 1.6 $gSO_4^{2-}gVSS^{-1}.d^{-1}$) in the UASB reactor during phase VII, as shown in Figure 7. During this phase, there was higher COD consumption and lower dispersion in the VFA and alkalinity values; that is, the reactor operation was more stable. This is because in this new configuration, no biomass washout was observed and the bacterial population distribution throughout the UASB reactor was homogenized, as indicated by a higher VSS content in port b during this phase (Figure 8). Despite a shorter residence time, better mixing conditions coupled with the presence of a solid enabled the presence of an SRB population larger than 10^9 free cells.mL^{-1} (Figure 6A) and therefore much larger sulfate removal efficiencies, which reached 97% (Figure 3), corresponding to a specific sulfate reducing activity rate of 4.8 $gSO_4^{2-}gVSS^{-1}.d^{-1}$ already in phase I (Table 3). Therefore, the FBR configuration favored sulfidogensis because competition by fermentative bacteria was reduced, which was likely due to better mass transfer with a better utilization of the substrate (Figures 6A and 6B).

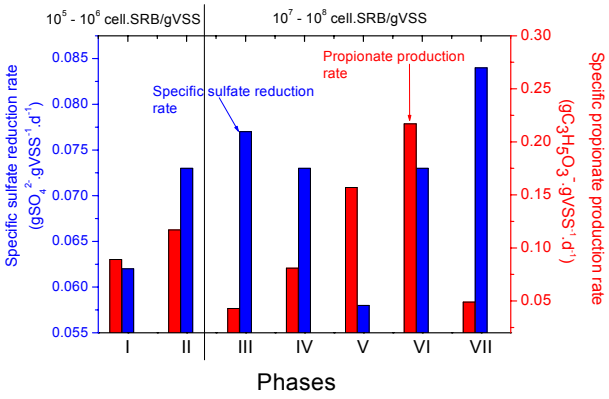

Figure 7: Values of specific sulfate-reduction and propionate production rates in the UASB reactor. Phase VI is characterized by a change in both flow rate and lactate concentration.

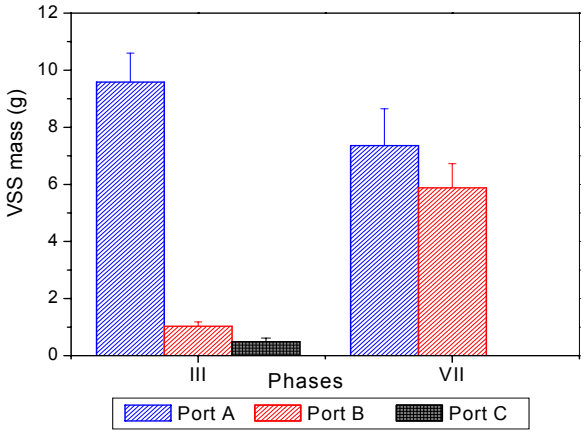

Figure 8: Biomass profile in the UASB reactor (ports a, b and c) during phase III (no recirculation) and VII (with recirculation). Port *c* during phase VII was utilized for biomass recirculation.

Sulfate Reduction in the Presence of Pure Glycerol as Substrate

As the FBR presented the best performance during sulfate reduction in the presence of lactate as a substrate, it was selected for further testing with a different carbon source, glycerol, which is a potentially inexpensive substrate for sulfate reduction (Kolesárová *et al*., 2011). Sulfate reduction with glycerol showed 90% efficiencies and average residual sulfate concentrations of 300 $mg.L^{-1}$ (Figure 3A). The average specific sulfate reduction rate (0.172±0.010

$gSO_4^{2-}.gVSS^{-1}.d^{-1}$) was similar to that measured when lactate was the only carbon source (0.191 ± 0.016 $gSO_4^{2-}.gVSS^{-1}.d^{-1}$), whereas the average specific COD removal rate (0.314 ± 0.071 $gCOD.gVSS^{-1}.d^{-1}$) was superior to the highest rate observed with lactate (0.266 ± 0.027 $gCOD.gVSS^{-1}.d^{-1}$), as depicted in Table 3. As glycerol became the substrate (phase VI), there was a remarkable reduction in both VFA and alkalinity values. Also, there was a decrease in acetate concentration, along with the appearance of butyrate (Figure 9) in the effluent, suggesting glycerol fermentation (Leja *et al.*, 2011), as will be discussed subsequently.

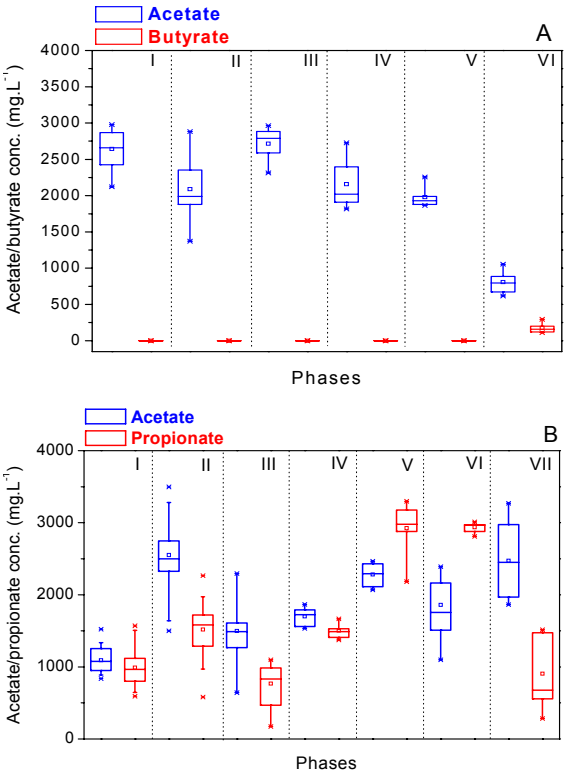

Figure 9: Acetate, butyrate and propionate profiles in the FBR (a) and the UASB reactor (b). Details on the different phases are depicted in Figure 2.

DISCUSSION

The main metabolic pathways accounting for sulfate reduction and organic matter oxidation in both the UASB and the FBR can be assessed by analyzing the relationship between microbial diversity and VFA profiles. Figure 10 depicts a summary of such outcomes: the microorganisms identified in the biomass during lactate oxidation along with both VFA profiles in both reactor effluents and the proposed metabolic pathways.

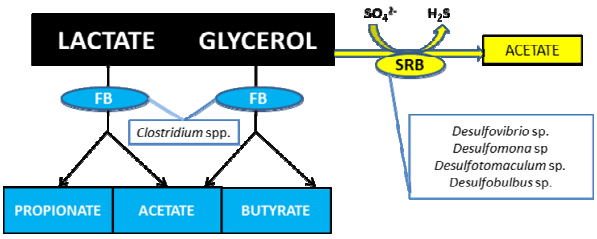

Figure 10: Main metabolic pathways developed during continuous sulfate removal in UASB and FBR during lactate and glycerol degradation. FB - Fermenting Bacteria; SRB - Sulfate Reducing Bacteria.

Although lactate was not observed in the UASB reactor effluent, it took 220 days for the SRB population to reach 10^8–10^9 cells.mL^{-1} (Figure 6B), and thus a sulfate reduction efficiency of 66% was attained (Bertolino *et al.*, 2012). That afforded the proposal of two main metabolic pathways in the reactor: (i) incomplete lactate oxidation to acetate during sulfate reduction by SRB (reaction 1) and (ii) lactate fermentation to both acetate and propionate by fermentative bacteria (FB), such as *Clostridium* (reaction 2) (Bertolino *et al.*, 2012):

$$2\ C_3H_5O_3^- + SO_4^{2-} \rightarrow 2\ C_2H_3O_2^- + HS^- + 2\ HCO_3^- + H^+ \quad (-160.1\ kJ) \tag{1}$$

$$3\ C_3H_5O_3^- \rightarrow C_2H_3O_2^- + 2\ C_3H_5O_2^- + HCO_3^- + H^+ \quad (-169.7\ kJ) \tag{2}$$

Such a competition between SRB and FB was implied by the propionate profile observed in the UASB reactor (Figure 9). As the organic loading rate increased from 3.55 ± 0.25 g.L^{-1}.d^{-1} (phase III) to 5.89 ± 0.48 g.L^{-1}.d^{-1} (phases V), there was an increase in the specific propionate production rate (from 0.043 ± 0.018 g.gVSS^{-1}.d^{-1} to 0.157 ± 0.019 g.gVSS^{-1}.d^{-1}), that is, larger fermentative activity. That resulted in a lower specific sulfate reduction rate (which decreased from 0.077 $gSO_4^{2-}.gVSS^{-1}.d^{-1}$ to 0.057 $gSO_4^{2-}.gVSS^{-1}.d^{-1}$), as shown in Figure 7, as well as a low utilization of the carbon source (Figure 6B). Such outcomes could be credited to lactate fermentation as the dominant pathway at high COD/sulfate ratios (Oyekola *et al.*, 2009), which was similar to that applied during phase V. Biomass recirculation in the UASB reactor (phase VII) led to an increase in the specific sulfate-reduction rate (Figure 7) and also a decrease in propionate production (Figure 9) and thus lower

fermentative activity (Beaulieu *et al*., 2000; Omil *et al*., 1998).

Fluidization and SRB retention by carbon particles in the FBR strongly reduced (or avoided) competition between SRB and FB because propionate was absent in the reactor effluent when lactate was the carbon source (Figure 9). Only acetate (2000 $mg.L^{-1}$ to 2750 $mg.L^{-1}$) was observed among the analyzed VFA, indicating incomplete lactate oxidation during sulfate reduction (reaction 1). Sulfate reduction as the predominant metabolic pathway in the FBR was further supported by a mass balance for substrate utilization, which confirmed that the entire inlet COD was converted only to acetate (Figure 9); therefore, Equation (1) solely accounted for acetate production (Figure 6A). Sulfate reduction was lower only in those phases where the $COD.sulfate^{-1}$ ratio was below 2 (II and IV); therefore, the organic substrate was limiting according to Equation (1). For instance, during phase I, a 97±2% sulfate reduction was observed for an OLR of 12.34±0.98 $gCOD.L^{-1}.d^{-1}$ ($COD.sulfate^{-1}$ mass ratio > 2.5), as compared to 78±10% when the OLR was 8.7±0.63 $g.L^{-1}.d^{-1}$ ($COD.sulfate^{-1}$ mass ratio of 1.8) in phase II. As the biomass concentration leveled out at 18.0 $gVSS.L^{-1}$ (from phase III, onwards), the specific sulfate reduction rate was 0.084±0.014 $gSO_4^{2-}.gVSS^{-1}.d^{-1}$ in the UASB reactor (Figure 7), which is one order of magnitude smaller than that observed in the FBR (0.191±0.016 $gSO_4^{2-}.gVSS^{-1}.d^{-1}$, Table 3), for which the biomass concentration was 24.5 $gVSS.L^{-1}$, considering free and attached (to activated charcoal) cells.

The incomplete oxidation of lactate to acetate (Equation (1)) is further supported by the absence of acetoclastic-SRB (Rodrigues, 2012), which explained acetate accumulation in the reactor effluent (Figure 9). It must be emphasized that incomplete substrate oxidation also affected the $COD.sulfate^{-1}$ ratio required in the system. According to Equation (1), during the reduction of 2.0 $g.L^{-1}$ (21 $mmol.L^{-1}$) sulfate, 4.0 $gCOD.L^{-1}$ (42 mmol Lactate.L^{-1}) would be required, following the incomplete oxidation pathway; therefore, the required $COD.sulfate^{-1}$ ratio should be 2 instead of 0.67 (required when complete substrate oxidation is predominant). This explained the largest sulfate reduction yields observed for $COD.sulfate^{-1}$ mass ratios above 2.5 (Table 3) in both FB and UASB reactors (as shown in Figures 2 and 3).

The behavior of the FBR was different when glycerol replaced lactate. Although the fermentative activity was considered negligible when lactate was utilized in the FBR with glycerol as the carbon source (phase VI), butyrate (150 $mg.L^{-1}$) was detected in the reactor effluent along with acetate (809±143 $mg.L^{-1}$). Such an outcome suggested the onset of fermentative activity in the FBR (Drożdżyńska *et al*., 2011) due to the presence of *Clostridium sp,* which was identified in all phases of FBR operation (Rodrigues, 2012). When metabolizing glycerol, some *Clostridium* species were produced in addition to acetate and butyrate, 1,3-Propanediol (Biebl and Spröer, 2002; Drożdżyńska *et al*., 2011), which likely did not accumulate in the system because it is also utilized by SRB (Qatibi, 1990).

A metabolic pathway for the oxidation of glycerol during sulfate reduction by a mixed SRB population was hypothesized by Dinkel *et al*. (2007) and was presented in reaction 3. It predicted that alkalinity should be lower than that produced during lactate degradation (reaction 1), explaining the experimental results achieved in the FBR (Figure 4A). From the stoichiometry of Equation (3) and the residual sulfate concentration (3.0 $mmol.L^{-1}$), the amount of acetate produced during the reduction of 18 $mmol.L^{-1}$ of sulfate can be estimated as 7.2 $mmol.L^{-1}$, which was lower than the measured acetate concentration (13.8 $mmol.L^{-1}$). Following such observations, it is herein suggested that roughly 50% of the acetate produced was due to glycerol oxidation by SRB (particularly *Desulfovibrio spp.*) during sulfate reduction, while the other 50% can be related to glycerol fermentation by *Clostridium* ssp. Such an outcome suggests that glycerol was not as easily degradable as lactate because of the conditions in the FBR. Indeed, the maximum specific growth rate of SRB on a glycerol-based medium was reported as 0.056 h^{-1} (Dinkel *et al*., 2010), which is one order of magnitude lower than that reported for SRB growth on lactate (Zellner *et al*., 1994).

$$\begin{aligned} &C_3H_8O_3 + 1.25\ SO_4^{2-} \rightarrow 0.5\ C_2H_3O_2^- \\ &+1.5\ H_2CO_3 + 0.5\ HCO_3^- + 1.25\ HS^- \\ &+0.75OH^- + 0.25H_2O \quad (-424.5\ kJ) \end{aligned} \tag{3}$$

Acetate buildup is reported as a drawback in high-rate sulfate-reducing reactors (Celis-García *et al*., 2007; Kaksonen *et al*., 2003; Nagpal *et al*., 2000) because the amount of residual COD in the reactor effluent requires downstream treatment. In this regard, the present study has demonstrated that sulfate reduction in the presence of glycerol as an organic substrate produced a smaller residual COD (1700 $mg.L^{-1}$) than that observed with lactate (2500 $mg.L^{-1}$ $C_2H_3O_2^-$) at the same $COD.sulfate^{-1}$ mass ratio (2.5),

which can be explained by the stoichometry of Equations (1) and (3). Such values are even smaller than those produced (2660 $mg.L^{-1}$ $C_2H_3O_2^-$) when ethanol (utilized in industrial scale sulfate-reducing plants) was applied as a carbon and electron source (Nagpal *et al*., 2000). The sulfide produced can be separated from acetate by either precipitation with transition metals (Fe, Cu, Ni) (Cao *et al*., 2009) or stripping by an inert gas (N_2 or CO_2), as proposed by Marre *et al*. (2004), or even by oxidizing to elemental sulfur (by Fe^{3+} or NO_3^-), as already utilized in industrial processes (Johnson *et al*., 2006). After H_2S removal, acetate can be degraded either aerobically or anaerobically, depending on the process configurations and feed water quality. Overall, as a by-product of the emerging biodiesel industry, crude glycerol may be foreseen as a cost-effective alternative to lactate and ethanol for sulfate reduction. Future work will focus on the application of crude glycerol for sulfate removal.

CONCLUSIONS

Mixing conditions play a key role during sulfidogenesis. Lactate fermentation by *Clostridium* spp. was an important metabolic pathway in a bench-scale UASB reactor treating 2.0 $g.L^{-1}.d^{-1}$ sulfate, without biomass recirculation (poor mixing conditions). An increase in the upflow velocity from 0.125 $m.h^{-1}$ to 1.75 $m.h^{-1}$ due to recirculation improved the biomass distribution in the reactor and thus the sulfate removal rate to 1.6 $gSO_4^{2-}.L^{-1}.d^{-1}$ (89% removal), but it decreased the propionate production rate to 0.88 $g.L^{-1}.d^{-1}$. Therefore, improved mixing conditions in the UASB reactor enhanced both substrate degradation and sulfate reduction, as opposed to substrate fermentation. In the fluidized bed reactor, good mass transfer conditions enabled the predominance of sulfate-reducing activity by incomplete-oxidizing SRB. When sulfate was not limiting (COD. $sulfate^{-1}$ mass ratios higher than 2), the sulfate removal rate varied between 4.7 $g.L^{-1}.d^{-1}$ and 5.1 $g.L^{-1}.d^{-1}$, which corresponds to sulfate removal efficiencies higher than 95%. The FBR was able to utilize pure glycerol as a carbon and electron source, producing sulfate reduction rates (0.172±0.010 $gSO_4^{2-}.gSSV^{-1}.d^{-1}$) similar to those observed with lactate (0.191±0.016 $gSO_4^{2-}.gSSV^{-1}.d^{-1}$). As a by-product of the biodiesel industry, glycerol can be a cost-effective option for sulfate reduction, leading to lower acetate concentrations (1700 $mg.L^{-1}$) when compared to lactate oxidation (2500 $mg.L^{-1}$).

ACKNOWLEDGEMENTS

The financial support from the funding agencies FINEP, FAPEMIG, CNPq, and CAPES, as well as Vale, is gratefully appreciated. The scholarships to S. M. Bertolino, S. F. Aquino, and V. A. Leão are especially acknowledged.

REFERENCES

Álvarez, J. A., Otero, L. and Lema, J. M., A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. Bioresource Technology 101, 1153 (2010).

APHA, Standard Methods for the Examination of Water and Wastewater. 21st Ed., (2005).

Barton, L. L., Sulfate-Reducing Bacteria. Biotechonology Handbooks, Vol. 8, Plenum Press, New York (1995).

Beaulieu, S., Zagury, G. J., Deschênes, L. and Samson, R., Bioactivation and bioaugmentation of a passive reactor for acid mine drainage treatment. Sixth International Conference on Environmental Issues and Management of Waste in Energy and Mineral Production, Calgary, Canada (2000).

Bertolino, S. M., Rodrigues, I. C. B., Guerra-Sá, R., Aquino, S. F. and Leão, V. A., Implications of volatile fatty acid profile on the metabolic pathway during continuous sulfate reduction. Journal of Environmental Management 103, 15 (2012).

Bhattacharya, S. K., Uberoi, V. and Dronamraju, M. M., Interaction between acetate fed sulfate reducers and methanogens. Water Research 30, 2239 (1996).

Biebl, H. and Spröer, C., Taxonomy of the glycerol fermenting clostridia and description of *Clostridium diolis sp. nov*. Systematic and Applied Microbiology 25, 491 (2002).

Cao, J., Zhang, G., Mao, Z., Fang, Z. and Yang, C., Precipitation of valuable metals from bioleaching solution by biogenic sulfides. Minerals Engineering 22, 289 (2009).

Celis-García, L. B., Razo-Flores, E. and Monroy, O., Performance of a down-flow fluidized bed reactor under sulfate reduction conditions using volatile fatty acids as electron donors. Biotechnology and Bioengineering 97, 771 (2007).

Dar, S., Kleerebezem, R., Stams, A., Kuenen, J. and Muyzer, G., Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. Applied Microbiology and Biotechnology 78, 1045 (2008).

Dinkel, V., Frechen, F., Dinkel, A., Smirnov, Y. and Kalyuzhnyi, S., Kinetics of anaerobic biodegradation of glycerol by sulfate-reducing bacteria. Applied Biochemistry and Microbiology 46, 712 (2010).

Drożdżyńska, A., Leja, K. and Czaczyk, K., Biotechnological production of 1,3-propanediol from crude glycerol. Journal of Biotechnology, Computational Biology and Bionanotechnology 92, 92 (2011).

Fountoulakis, M. S. and Manios, T., Enhanced methane and hydrogen production from municipal solid waste and agro-industrial by-products co-digested with crude glycerol. Bioresource Technology 100, 3043 (2009).

Harada, H., Uemura, S. and Momonoi, K., Interaction between sulfate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low strength wastes containing different levels of sulfate. Water Research 28, 355 (1994).

INAP, Treatment of sulphate in mine effluentes. International Network for Acid Prevention (2003).

Jacobs, P. and Pulles, W., Best practice guidelines for water resources protection in the South African mining industry. Department of Water Affairs and Forestry: Auckland Park, South Africa (2007).

Johnson, D. B., Sen, A. M., Kimura, S., Rowe, O. F. and Hallberg, K. B., Novel biosulfidogenic system for selective recovery of metals from acidic leach liquors and waste streams. Mineral Processing and Extractive Metallurgy (Trans. Inst. Min. Metall. Section C) 115, 19 (2006).

Kaksonen, A. H., Franzmann, P. and Puhakka, J. A., Performance and ethanol oxidation kinetics of a sulfate-reducing fluidized-bed reactor treating acidic metal-containing wastewater. Biodegradation 14, 207 (2003).

Kaksonen, A. H., Plumb, J. J., Franzmann, P. D. and Puhakka, J. A., Simple organic electron donors support diverse sulfate-reducing communities in fluidized-bed reactors treating acidic metal- and sulfate-containing wastewater. FEMS Microbiology Ecology 47, 279 (2004).

Kato, M. T., Field, J. A., Versteeg, P. and Lettinga, G., Feasibility of expanded granular sludge bed reactors for the anaerobic treatment of low-strength soluble wastewaters. Biotechnology and Bioengineering 44, 469 (1994).

Kolesárová, N., Hutňan, M., Bodík, I. and Špalková, V., Utilization of biodiesel by-products for biogas production. Journal of Biomedicine and Biotechnology 2011, 1 (2011).

Leja, K., Czaczyk, K. and Myszka, K., Biotechnological synthesis of 1,3-propanediol using *Clostridium ssp.* African Journal of Biotechnology 10, 11093 (2011).

Liamleam, W. and Annachhatre, A. P., Electron donors for biological sulfate reduction. Biotechnology Advances 25, 452 (2007).

Liu, Y., Xu, H.-L., Yang, S.-F. and Tay, J.-H., Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor. Water Research 37, 661 (2003).

Lopez, O., Sanguinetti, D., Bratty, M. and Kratochvil, D., Green technologies for sulphate and metal removal in mining and metallurgical effluents. Enviromine Conference. Santiago, Chile (2009).

Maree, J. P., Beer, M., Strydom, W. F., Christie, A. D. M. and Waanders, F. B., Neutralizing coal mine effluent with limestone to decrease metals and sulphate concentrations. Mine Water and the Environment 23, 81 (2004).

Mohan, S. V., Prasad, K. K., Rao, N. C., Bhaskar, Y. V., Babu, V. L., Rajagopal, D. and Sarma, P. N., Biological treatment of low-biodegradable composite chemical wastewater using upflow anaerobic sludge blanket (UASB) reactor. Journal of Scientific and Industrial Research 64, 771 (2005).

Nagpal, S., Chuichulcherm, S., Peeva, L. and Livingston, A., Microbial sulfate reduction in a liquid–solid fluidized bed reactor. Biotechnology and Bioengineering 70, 370 (2000).

Nielsen, P. H., Biofilm dynamics and kinetics during high-rate sulfate reduction under anaerobic conditions. Applied and Environmental Microbiology 53, 27 (1987).

Omil, F., Lens, P., Hulshoff Pol, L. W. and Lettinga, G., Effect of upward velocity and sulphide concentration on volatile fatty acid degradation in a sulphidogenic granular sludge. Process Biochemistry 31, 699 (1996).

Omil, F., Lens, P., Visser, A., Hulshoff Pol, L. W. and Lettinga, G., Long-term competition between sulfate reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids. Biotechnology and Bioengineering 57, 676 (1998).

Oyekola, O. O., van Hille, R. P. and Harrison, S. T. L., Study of anaerobic lactate metabolism under biosulfidogenic conditions. Water Research 43, 3345 (2009).

Qatibi, A. I., Fermentation du lactate, du glycerol et des diols par les bactéris sulfato-réductrices du genere *Desulfovibrio*. Docteur Thesis. Université de Provence (1990). (In French).

Rampinelli, L., Azevedo, R., Teixeira, M., Guerra-Sá, R. and Leão, V., A sulfate-reducing bacterium with unusual growing capacity in moderately acidic conditions. Biodegradation 19, 613 (2008).

Ren, N. Q., Chua, H., Chan, S. Y., Tsang, Y. F. and Sin, N., Effects of COD/SO_4^{2-} ratios on an acidogenic sulfate-reducing reactor. Industrial & Engineering Chemistry Research 46, 1661 (2007).

Rodrigues, I. C. B., Métodos moleculares aplicados a biotecnologias relacionadas ao ciclo do enxofre. Thesis. Universidade Federal de Ouro Preto (2012). (In Portuguese).

Sahinkaya, E., Özkaya, B., Kaksonen, A. H. and Puhakka, J. A., Sulfidogenic fluidized-bed treatment of metal-containing wastewater at low and high temperatures. Biotechnology and Bioengineering 96, 1064 (2007).

Schmidt, J. E. and Ahring, B. K., Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. Biotechnology and Bioengineering 49, 229 (1996).

Shayegan, J., Ghavipanjeh, F. and Mirjafari, P., The effect of influent COD and upward flow velocity on the behaviour of sulphate-reducing bacteria. Process Biochemistry 40, 2305 (2005).

Sheoran, A. S., Sheoran, V. and Choudhary, R. P., Bioremediation of acid-rock drainage by sulphate-reducing prokaryotes: A review. Minerals Engineering 23, 1073 (2010).

Speece, R. E., Anaerobic biotechnology for industrial wastewater treatment. Environmental Science & Technology 17, 416A (1983).

Velasco, A., Ramírez, M., Volke-Sepúlveda, T., González-Sánchez, A. and Revah, S., Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation. Journal of Hazardous Materials 151, 407 (2008).

WHO, Guidelines for drinking-water quality. Genebra (2011).

Yang, Y., Tsukahara, K. and Sawayama, S., Biodegradation and methane production from glycerol-containing synthetic wastes with fixed-bed bioreactor under mesophilic and thermophilic anaerobic conditions. Process Biochemistry 43, 362 (2008).

Zellner, G., Neudörfer, F. and Diekmann, H., Degradation of lactate by an anaerobic mixed culture in a fluidized-bed reactor. Water Research 28, 1337 (1994).

Zhao, Y., Ren, N. and Wang, A., Contributions of fermentative acidogenic bacteria and sulfate-reducing bacteria to lactate degradation and sulfate reduction. Chemosphere 72, 233 (2008).