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A successful strategy for start-up of a laboratory-scale UASB reactor treating sulfate-rich sugar cane vinasse

Ernesto L Barrera,[a*] Henri Spanjers,[b] Osvaldo Romero,[a] Elena Rosa[c] and Jo Dewulf[d]

Abstract

BACKGROUND: The aim of the present research was to provide a strategy for the start-up of upflow anaerobic sludge blanket (UASB) reactors treating sugar cane vinasse, with emphasis on the relevant sulfur compounds along the reactor height. To this end, a laboratory-scale UASB reactor was started up and its performance under the applied conditions was studied.

RESULTS: Biomass was able to assimilate organic loading rates from 3 to 10 kg chemical oxygen demand (COD) m_R^{-3} d^{-1}, reducing VFAs accumulation, while increasing biogas production rate (BPR) from 0.92 to 4.12 m^3 m_R^{-3} d^{-1}. The COD and sulfate (SO_4^{2-}) were mainly removed in the reactor bed, showing homogenous distribution in the blanket and settler zones. However, from bottom to top of the reactor, a slight decrease of total and free sulfide concentrations was observed, indicating that the sludge bed in the UASB reactor was exposed to H_2S concentrations higher than those measured in the reactor effluent.

CONCLUSIONS: During 54 days, the development of macroscopic granules, the COD removal efficiency, the BPR and the CH_4 content proved good indicators to monitor the start-up period. Further research will be needed for strategy validation in pilot and full-scale UASB reactors treating vinasse.

Keywords: reactor profiles; start-up; sulfur; UASB reactor; vinasse

INTRODUCTION

Vinasse is a by-product obtained after the distillation of fermented cane molasses to produce ethanol and is considered as a biodegradable wastewater suitable for anaerobic digestion (AD). In Cuba more than 1.3 million m^3 of vinasse is emitted each year (Cuban Ministry of the Cane Sugar Industry, 2014). However, vinasse has been considered as a sulfate-rich liquid substrate for AD, where high chemical oxygen demand (COD) and sulfate (SO_4^{2-}) concentrations coexist, stimulating sulfate reduction processes, and leading to reduced methane (CH_4) yield and operational difficulties (i.e. inhibition of AD and additional requirements for biogas cleaning) as a result of the produced sulfide in the gas and liquid phases.[1]

Upflow anaerobic sludge blanket (UASB) reactors are used widely in industrial and domestic wastewater treatment systems to reduce COD and to produce CH_4.[2] However, for efficient CH_4 production, strategies need to be developed for UASB reactor start-up.[3] The start-up period has been defined as the time taken for the development of the first macroscopic sludge granules from fresh dispersed inoculum.[4] Although general guidelines to ensure successful start-up of UASB reactors are well-documented,[2] specific strategies for start-up operation in UASB reactors fed with vinasse should be developed.

As a methodology for monitoring start-up operation, the sludge concentration profile over the reactor height and the COD degradation of an easily biodegradable and synthetic wastewater [i.e. mixture of volatile fatty acids (VFAs) and glucose], were investigated by Vadlani and Ramachandran.[5] They showed that specific uptake rates of acetic and butyric acids and COD removal rates can be improved with time by gradually increasing organic loading rate (OLR). The specific uptake rates and the active biomass concentration were found higher near the inlet compared to the remaining reactor volume fraction. Although this methodology for monitoring start-up operation was considered applicable for industrial wastewaters, it was not validated using vinasse.

Start-up operation of a UASB reactor inoculated with a non-adapted sludge and fed with vinasse was reported recently.[3] The

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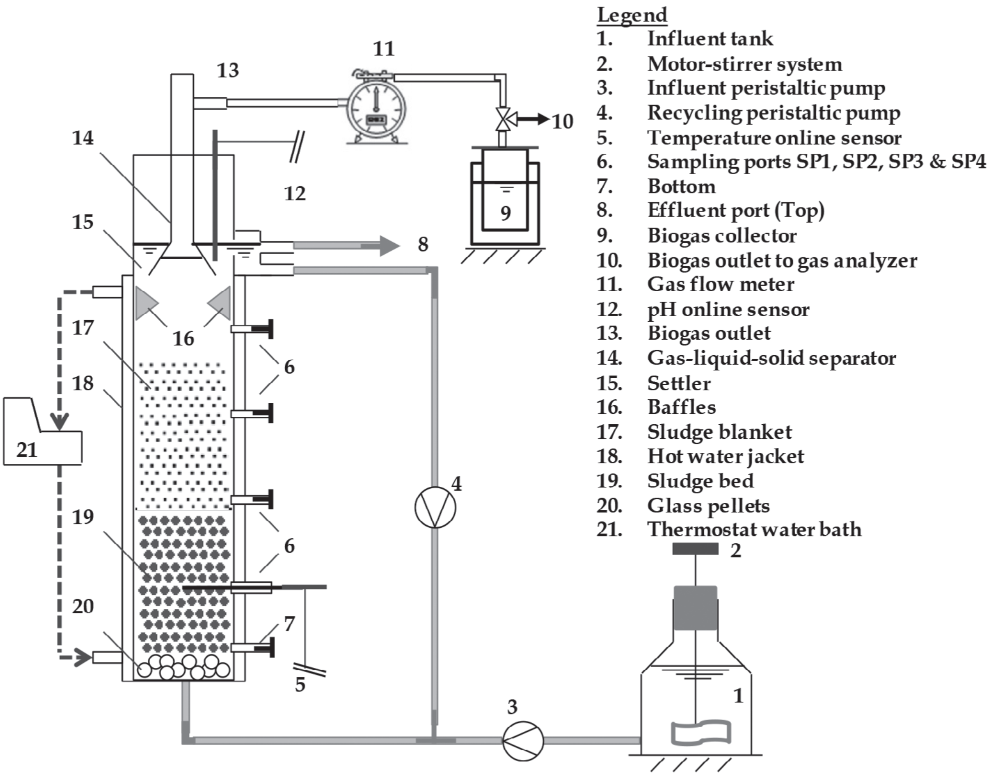

Figure 1. Schematic of the experimental set-up used in the evaluation of the start-up.

results demonstrated that >200 days can be required when using nonadapted sludge for start-up operation. Even though vinasse is a known sulfate-rich liquid substrate for anaerobic digestion, the sulfur compounds were neglected during their reactor start-up.

Therefore, the aim of the present work was to provide a strategy for the start-up of a laboratory-scale UASB reactor treating sulfate-rich sugar cane vinasse, with emphasis on the relevant sulfur compounds along the reactor height.

MATERIALS AND METHODS

Experimental set-up

The laboratory-scale UASB reactor with 3.5-L working volume (as the sum of the sludge bed, the sludge blanket and the settler volumes) consisted of an acrylic transparent column with internal diameter 8 cm and height 70 cm. The reactor was equipped with four sample ports: SP1, SP2, SP3 and SP4, placed along the reactor height at 14, 28, 42 and 56 cm from the inlet, respectively. Thirty percent of the reactor working volume (21 cm of the reactor height, between SP1 and SP2) was filled with sludge. SP1 was used only for measuring the sludge temperature ($35 \pm 2\,^{\circ}$C). Sludge temperature and effluent pH were measured online by a Data Acquisition System. The schematic is shown in Fig. 1.

Seed sludge

A dispersed sludge (particle size ≤ 1 mm) was collected from a 3600 m^3 UASB reactor treating vinasse (Heriberto Duquesne, Santa Clara, Cuba), with design upflow velocity of 0.2 m h^{-1}. The specific methanogenic activity (SMA) of the sludge and the volatile suspended solids (VSS) concentration were 0.14 gCH$_4$-COD gSVSS^{-1} d^{-1} and 9.82 gVSS L^{-1}, respectively.

Influent characteristics

A composite sample of 60 L of vinasse was obtained during a normal operating day from a distillery plant in Sancti Spiritus (similar distillation process as in Heriberto Duquesne, Santa Clara, Cuba), Cuba and immediately stored at $-20\,^{\circ}$C to avoid biodegradation. No additional nutrients were added given the presence of micro- and macro-elements in the fed vinasse; the characteristics of the raw vinasse used for preparing the reactor influent are shown in Table 1.

The start-up strategy

The whole start-up regime has been considered to consist of five periods,[6] namely: (i) incubation/batch feed period; (ii) adaptation period I (initial period); (iii) adaptation period II (intermediate period); (iv) adaptation period III (granule formation period); and (v) start-up period (granule development in size and shape). In the present work the sludge was collected from a full-scale UASB reactor treating vinasse, so it was considered as a well-adapted inoculum and thus requiring only the fifth period of the start-up regime (the start-up period).[6] This was the concept herein for the start-up of the UASB reactor fed with vinasse.

The guideline for start-up of UASB reactors was taken as the basis[2] for increasing the OLR step-by-step (by 30%) and preserving the ratio of volatile fatty acids (VFAs) to total alkalinity (TA) between 0.1 to 0.2, before applying a higher OLR. This was combined with the approach of Vadlani and Ramachandran,[5] which monitors VSS concentration profiles along the reactor height, for understanding reactor behaviour through the distribution of the active biomass. Concentration profiles for COD, sulfate and sulfides were studied along the reactor height [reactor bottom (RB), SP2, SP3, SP4 and reactor top (RT)], in addition to the aforementioned strategies, to follow the organic matter degradation and the

Table 1. Characteristics of the raw vinasse ($n = 3$) used during start-up

Parameter	Units	Average	Parameter	Units	Average
COD	kgCOD m^{-3}	52.48	Na^+	kmol m^{-3}	0.04
TOC	kmolC m^{-3}	15.56	K^+	kmol m^{-3}	0.09
TKN	kmolN m^{-3}	0.037	Ca^{2+}	kmol m^{-3}	0.04
NH_4^+-N	kmolN m^{-3}	0.00025	Mg^{2+}	kmol m^{-3}	0.02
TS	kg m^{-3}	46.88	Mn^{2+}	kmol m^{-3}	n.d
VS	kg m^{-3}	36.81	Zn^{2+}	kmol m^{-3}	n.d
FS	kg m^{-3}	10.07	Total cations	kmol m^{-3}	0.18
TSS	kg m^{-3}	4.15	SO_4^{2-}	kmol m^{-3}	0.02
VSS	kg m^{-3}	3.11	NO_2^-	kmol m^{-3}	n.d
FSS	kg m^{-3}	1.05	NO_3^-	kmol m^{-3}	n.d
Sugars	kg COD m^{-3}	32.72	PO_4^{3-}	kmol m^{-3}	n.d
Lipids	kgCOD m^{-3}	0.19	Cl^-	kmol m^{-3}	0.04
Proteins	kgCOD m^{-3}	4.77	Total anions	kmol m^{-3}	0.06
VFAs (acetic)	kgCOD m^{-3}	1.12			
pH	–	4.77			

n.d., not detected.

Table 2. Start-up operating parameters

Start-up phases	Duration (d)	OLR (kgCOD m_R^{-3} d^{-1})	SLR (kg SO_4^{2-} m_R^{-3} d^{-1})	COD (kgCOD m^{-3})	V_{up} (10^{-3} m h^{-1})	Q_{in} (10^{-3} $m^3 d^{-1}$)	R_r	HRT (d)
I	0–7	3.03	0.10	4.32	22	2.640	0	1.32
TP	8–12	4.25	0.12	5.63	47	2.640	1.15	1.32
II	13–26	5.66	0.17	7.37	52	2.688	1.31	1.30
III	27–37	7.34	0.22	9.31	55	2.760	1.41	1.26
IV	38–55	10.01	0.28	12.69	60	2.760	1.62	1.26

TP, transition period; OLR, organic loading rate; SLR, sulfate loading rate; COD, chemical oxygen demand; V_{up}, upward velocity; Q_{in}, influent flow; R_r, recirculation ratio; and HRT, hydraulic retention time.

sulfate reduction process along the reactor height during the AD of vinasse. The concentrations of COD, sulfate and sulfides at the RB, were calculated by mass balances from their measured concentrations in the fed and the recirculated vinasse and the measured flows (recycled and influent). Free and dissolved hydrogen sulfide (H_2S) concentrations also were monitored to understand a possible process inhibition.

The main steps and operating condition during the start-up phases are described below (Table 2):

Phase I: An OLR of 3.03 kgCOD m_R^{-3} d^{-1}, (where mR^{-3} refers to cubic meter of the UASB reactor) was applied by diluting the raw vinasse with tap water to 4.32 kgCOD m^{-3}. An upflow velocity ($V_{up} = 0.022$ m h^{-1}) below the design values was used to avoid biomass washout, as the sludge was observed to be disperse. As pH of the raw vinasse was 4.77, the influent pH was adjusted to 6.5 by adding NaOH (6 mol L^{-1}). COD, VSS, sulfate and sulfide concentration profiles were monitored along the reactor height in order to evaluate the level of assimilation of the OLR using as criteria: (i) the achievement of COD and sulfate removal efficiencies similar to or higher than the previous step and near to the values obtained for UASB reactors treating sulfate-rich wastewater[7,8]; (ii) the degradation capacities of biomass; and (iii) the possible sulfide inhibition occurring when sulfate loading rate (SLR) increased.

Transition period (TP): After seven days, a recirculation ratio (R_r) of 1.15 was applied to increase V_{up} to 0.047 m h^{-1}, using the alkalinity generated inside the reactor for neutralization. The addition of NaOH (6 mol L^{-1}) was stopped. After one HRT (Day 9), the OLR was increased by 30% (4.25 kgCOD m_R^{-3} d^{-1}). This period was not evaluated as it was considered a transition interval to increase V_{up} with effluent recirculation, causing dilution of the influent to COD concentration to values below the applied in Phase I. A further discussion on this period is provided in the Reactor performance section below section 3.1.

Phase II: The COD concentration was increased to 7.37 kgCOD m^{-3} by reducing the vinasse dilution further. Consequently, OLR increased by 30% (5.66 kgCOD m_R^{-3} d^{-1}) with respect to TP. Influent flow rate was kept around constant, whereas the recirculation flow increased to obtain an R_r of 1.31 and V_{up} of 0.052 m h^{-1}. COD, VSS, sulfate and sulfide concentration profiles also were monitored along the reactor height.

Phase III: A 30% increase in the OLR with respect to Phase II was applied here. Both influent and recirculation flow rates were increased to reach an R_r and V_{up} of 1.41 and 0.055 m h^{-1}, respectively. COD, VSS, sulfate and sulfide concentration profiles again were monitored at the end of this phase to verify the correct assimilation of the increased OLR.

Phase IV: The final 30% increase in the OLR was applied by increasing COD concentration to 12.69 kgCOD m^{-3}. R_r and V_{up} values of 1.62 and 0.060 m h^{-1}, respectively, were obtained by increasing the recirculation flow rate. COD, VSS, sulfate and sulfide concentration profiles also were monitored at the end of this phase to verify the correct assimilation of the increased OLR.

It should be noted that HRT was kept approximately constant in correspondence with Q_{in}, whereas V_{up}, OLR, COD and SLR were gradually increased during the start-up phases (Table 2). For assessing the start-up performance of the UASB reactor, the CH_4 and sulfur yield were evaluated as in Barrera et al.[1]; and a specific methanogenic activity test for the sludge was carried out and linked with operating parameters such as biogas production rate (BPR), biogas composition, COD removal efficiency (R-COD) and sulfate removal efficiency (R-SO_4^{2-}).

Chemical analysis

In the gas phase, CH_4 and CO_2 concentrations were measured by means of a Pronova (SSM 6000 Classic, Berlin, Germany) gas analyzer equipped with the appropriate sensors. Gas phase H_2S concentration was measured by bubbling the biogas through a zinc acetate solution (0.07 mol L^{-1}), which was subsequently titrated using standard iodometric procedure.

A volume of 60–80 mL was sampled from the inlet, SP2, SP3 and SP4, and the outlet. The sample was filtered to determine VSS. Sulfates were analyzed by a turbidimetric method at 420 nm wavelength using a UV spectrophotometer (RAYLEIGH, UV-1601, Beijing, China). For this purpose, samples were incinerated and ashes were dissolved in HCl 1:1 (acid: distilled water) in order to avoid colour interferences. The close reflux titrimetric method (dichromate method) was used for COD determinations (total and soluble), subtracting H_2S COD. Total dissolved sulfides were determined by the iodometric method. To avoid sulfide loss during sample filtration, pH was raised to 10 by adding a few drops of NaOH (6 mol L^{-1}).[9] Interfering substances (thiosulfate, sulfite or organic matter) were removed by adding zinc acetate (0.7 mol L^{-1}) to precipitate sulfide as ZnS. Finally, samples were filtered and the retentate was titrated to determine the sulfide concentration. Free H_2S concentrations in the liquid phase were calculated using the total dissolved sulfide concentrations, the pKa values and the pH. Values for TA and VFAS were measured daily by titration to a pH of 5.00 and 4.40, respectively. Standard methods were followed in all cases.[10] The average value of three replicates (at least) was used in all cases.

Specific methanogenic activities test

The SMA of the sludge was measured at the end of the experimental phases, following Jiménez et al.[11] It was calculated from the slope of the accumulated CH_4 production curve over the first five to six days, divided by the amount of VSS introduced in the bottle, using the proper conversion factor to report it as gCOD-CH_4 $gVSS^{-1}$ d^{-1}.

RESULTS AND DISCUSSION

Reactor performance

The BPR, VFAs concentration, VFAs:TA ratio, pH and reactor temperature were monitored to evaluate the performance of the UASB reactor during the start-up phases (I, II, III and IV) (Fig. 2). The BPR increased with time showing average values of 0.92 ± 0.05, 2.14 ± 0.03, 3.13 ± 0.05 and 4.12 ± 0.19 m^3 m_R^{-3} d^{-1} during the last four days (∼3HRT) of the start-up phases, indicating steady-state conditions (variations <5%).[12]

A VFAs:TA ratio of >0.8 may inhibit methanogenic archaea, whereas ratios between 0.3 and 0.4 indicate an unstable system, and from 0.1 to 0.2 are known as favourable for increasing OLR in UASB reactors.[3,13] During the last four days of phases I, II, III and IV herein, the average VFAs:TA ratios in the effluent were 0.22 ± 0.02, 0.17 ± 0.02, 0.16 ± 0.00 and 0.25 ± 0.01, respectively, which can be considered as stable conditions (Fig. 2). Only at the beginning of phases I and II did the applied OLR cause VFAs:TA ratios of >0.8 but the ratios were drastically reduced after five days, and the biogas production remained increasing. Maximum VFAs concentrations of 17 and 13 meq L^{-1} at days 1 and 15, respectively, were found to be above the recommended values of 8 meq L^{-1};[2] being 50% lower than values reported.[3] Although the reactor pH was accurately controlled during those days, as recommended by Hulshoff Pol,[2] a pH drop to 6.4 was observed. This pH value was promptly recovered at Day 16, suggesting that the biomass was able to degrade the excess of VFAs and to generate the required alkalinity. After five days the VFAs concentration decreased to recommended values for start-up operation.[2] During the transition period, a stable VFAs:TA ratio (0.22 ± 0.02) was observed, with no accumulation of VFAs, together with an increase in the BPR. For that reason, it was considered as a transition period where the reactor easily assimilated the applied OLR, allowing switchover to the next phase.

Profiles along the reactor height

Soluble COD and VSS profiles

Understanding the reactor's behaviour from reactor concentration profiles was a major focus of this research. Soluble COD (COD_s) and VSS profiles are shown in Fig. 3. Although the OLR was gradually increased during phases I, II, III and IV, removal efficiencies of 72.8, 88.8, 80.0 and 78.0%, respectively, were achieved. It was demonstrated that the biomass was able to assimilate the increasing OLR, suggesting an increase of microbial activity and the involvement of higher sludge bed zones in the degradation of the organic matter. Most of the COD was removed in the lower 40% of the reactor working volume (at SP2), being 61.2% in Phase I and 78.0% in Phase IV (Fig. 3). In all phases, despite the increasing OLR, the COD remained approximately constant at SP2, SP3, SP4 and RT, indicating that COD distribution became homogenous after the sludge bed (after SP2), because of the reduction of VSS concentration along the reactor height (after SP2). From these results, it should be noted that 60% of the UASB reactor volume (blanket and settler zones: from SP2 to SP4) is not intended for organic matter removal. Although the biomass in the sludge blanket was active, very low biomass concentrations (compared to sludge bed) have been reported for this zone, leading to poor degradation of substrates.[14] This aspect might result in over-design of anaerobic reactors and should be carefully studied in future work, looking, for example, for alternative reactor configuration.

The VSS profiles of the UASB reactor, including RB, SP2, SP3, SP4 and RT, also are shown in Fig. 3. As a result of the OLR increments, sludge VSS concentration in the RB increased from 11 to 45 gVSS L^{-1}, suggesting that biomass became active to degrade the increased OLR. These results agreed with values (49 gVSS L^{-1}) reported by Barros *et al.*,[3] after start-up of UASB reactors fed with vinasse. The VSS concentration profiles remained at<0.2 gVSS L^{-1} along the reactor height, indicating a lower biomass washout than the experiment reported by Vadlani and Ramachandran (VSS concentration of 0.38 gVSS L^{-1}),[5] for the start-up of a laboratory-scale UASB reactor fed with a synthetic mixed-acid waste at similar OLR.

Sulfate, total dissolved sulfide and free H_2S profiles

Homogenous distribution of sulfate concentrations over SP2, SP3, SP4 and RT was found during all phases (Fig. 4), whereas R-SO_4^{2-} increased from 23.8 to 66.3% from phases I to IV. From the reduced

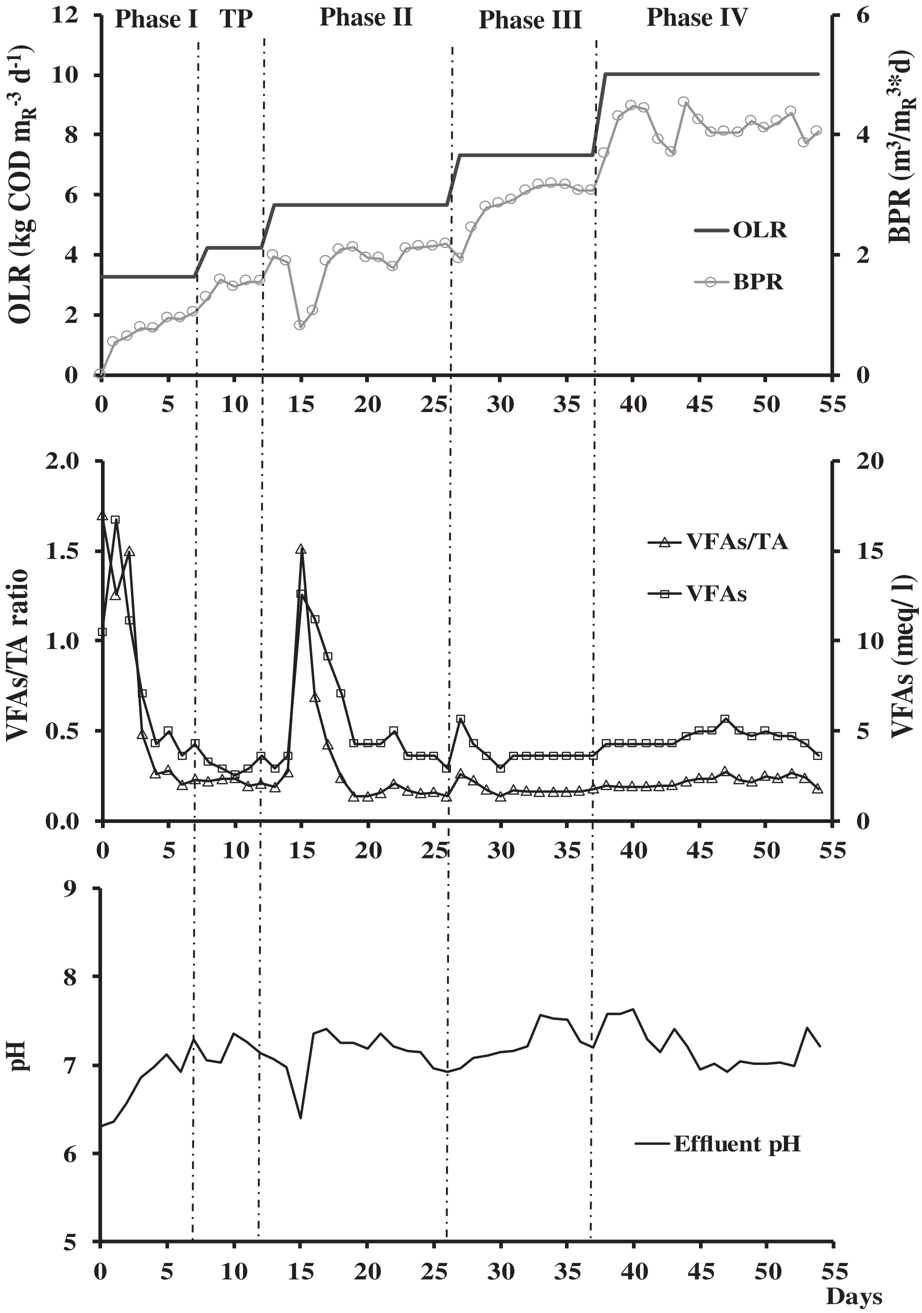

Figure 2. Start-up performance of the UASB reactor during phases I, II, III and IV. TP, transfer period; OLR, organic loading rate;, BPR, biogas production rate; VFAs, volatile fatty acids; TA, total alkalinity.

sulfate it was deduced that 1.3 to 2% of the removed COD was used by sulfate reducing bacteria (SRB) to produce H_2S. These low values (with respect to the COD used by methanogens) were attributed to sulfate limitation in the influent vinasse. Consequently, total dissolved and free sulfides always were found to be maximal at SP2 (Fig. 4), which is attributed to the consumption of sulfates in the lower 40% of the reactor working volume. Sulfides in RB came from the recirculated effluent that was mixed with the influent vinasse entering the reactor.

The slight decrease of total and free sulfide concentrations along the reactor height was attributed to the mass transfer of H_2S between the liquid and gas phases. This shows that the sludge bed in UASB reactors fed with sulfate-rich liquid substrates are exposed to H_2S concentrations higher than those measured in the reactor effluent. Total dissolved and free H_2S concentrations of >564 mgS L^{-1} and 150 mgS L^{-1}, respectively, have been reported to inhibit 50% the anaerobic consortia.[15] For that reason, in the present study during the start-up of a UASB reactor fed with vinasse at a SO_4^{2-}:COD ratio of ≈ 0.03, the H_2S concentrations (<10 mgS L^{-1}) were not expected to cause inhibition of the anaerobic digestion processes. However, higher SO_4^{2-}:COD ratios can be found in very high strength vinasse (COD concentrations >50 kgCOD m^{-3}), ranging from 0.10 to 0.22,[16,17] where higher sulfide concentrations can occur.[1] In those cases, the UASB reactor should be carefully monitored, especially in the sludge bed zone where higher sulfide concentrations can be found.

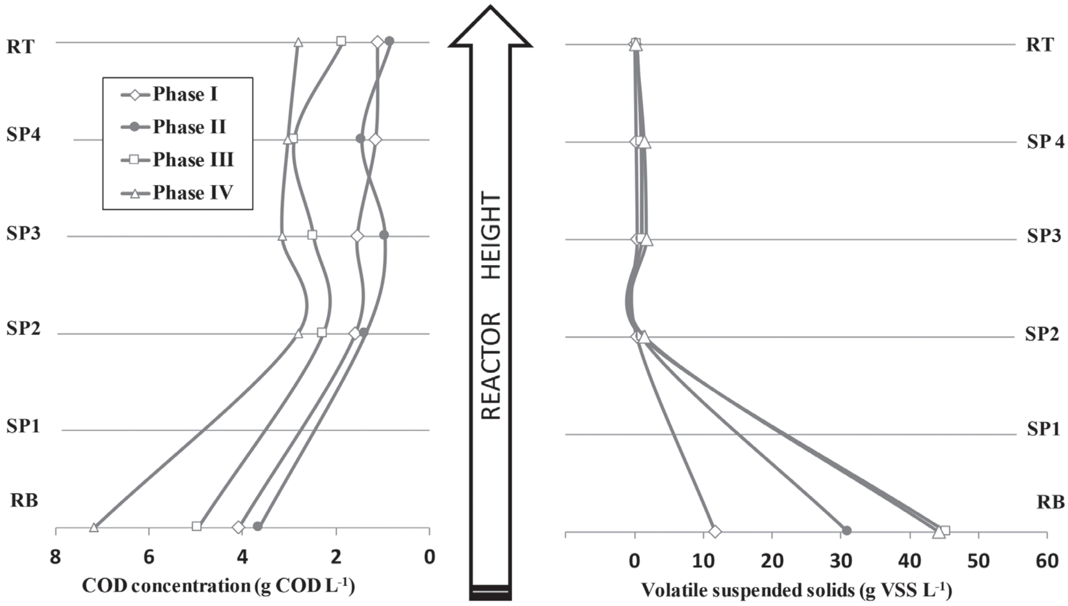

Figure 3. Soluble COD and VSS profiles along the reactor height during phases I, II, III and IV. Sample ports SP1, SP2, SP3 and SP4, reactor top (RT) and reactor bottom (RB).

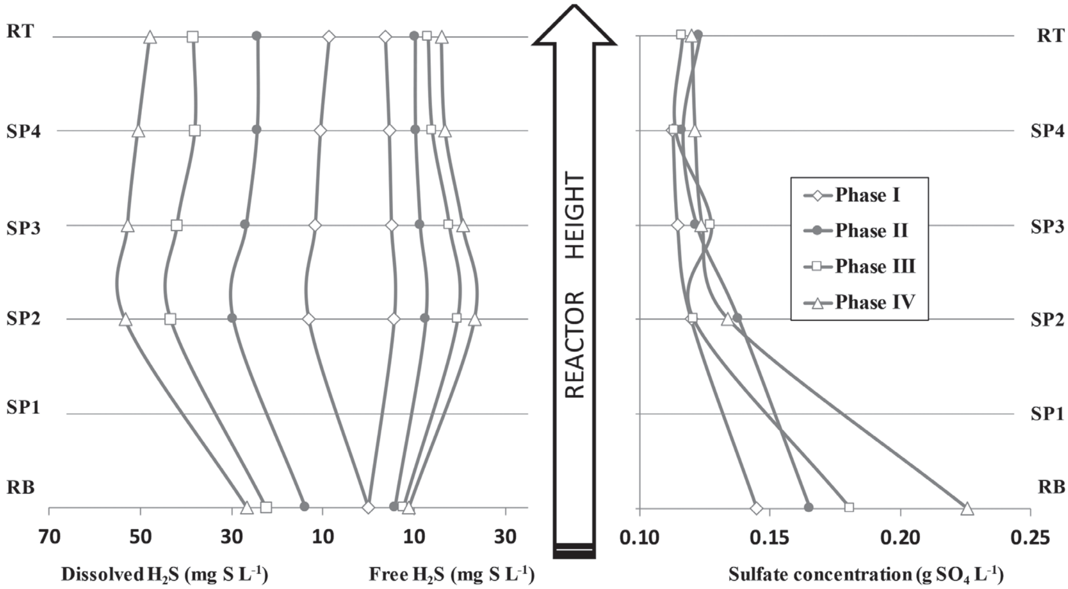

Figure 4. Sulfate, total dissolved and free sulfide profiles during phases I, II, III and IV. Sample ports SP1, SP2, SP3 and SP4, reactor top (RT) and reactor bottom (RB).

Performance parameters during start-up

The performance parameters during start-up are summarized in Table 3, showing CH_4 and S yields, SMA of the sludge, maximum granule size, R-COD, R-SO_4^{2-}, BPR and biogas composition (CH_4, CO_2 and H_2S) for phases I, II, III and IV.

At the end of Phase I, the SMA increased (0.19 gCOD-CH_4 $gVSS^{-1}$ d^{-1}) with respect to the inoculum (0.14 gCOD-CH_4 $gVSS^{-1}$ d^{-1}) collected from the full-scale UASB reactor. A CH_4 yield of 244.3 mL CH_4 $(gCOD\ removed)^{-1}$ d^{-1} (~70% of the theoretical value), and a very poor S yield of 80.4 mgS $(gSO4^{2-})$ (~24% of the theoretical value), suggested that methanogens were able to assimilate the applied OLR (R-COD ~73%), whereas SRB were less active, degrading only 23.8% of the influent sulfate. In addition, the granulation process started, developing from the dispersed sludge collected to granular sludge with a maximum size of 2 mm (Table 3).

In Phase II, a slight increase in the CH_4 yield (with respect to Phase I) to approximately 250 mL CH_4 $(gCOD\ removed)^{-1}$ d^{-1} was observed whereas the SMA also increased slightly from 0.19 to 0.21 gCOD-CH_4 $gVSS^{-1}$ d^{-1}. The S yield and the R-SO_4^{2-} increased to 151.1 mgS $(gSO_4^{2-})^{-1}$ and 44.2%, respectively, suggesting that SRB became adapted, becoming active and using a higher COD fraction for sulfide production (Table 3).

During Phase III, adaptation of SRB improved with respect to Phase II, allowing removal of the applied SLR, increasing the R-SO_4^{2-} by 13%, and the S yield by 44.1 mgS $(gSO_4^{2-})^{-1}$. In spite of that, a higher CH_4 yield also was observed [331.4 mL CH_4 (gCOD

Table 3. Summary of the performance parameters during the UASB reactor start-up

	CH_4 yield	Sulfur yield	Sludge SMA	Granule max. size	R-COD efficiency	R-SO_4^{2-} efficiency	BPR	Biogas composition		
Start-up Phases	$mLCH_4$ (g COD removed)$^{-1}$ d^{-1}	mgS (g SO_4^{2-})$^{-1}$	gCOD-CH_4 gVSS d^{-1}	mm	%	%	m^3 m_R^{-3} d^{-1}	%CH_4	%CO_2	%H_2S
I	244.3	80.4	0.19	2	72.8	23.8	0.92	59.3	33.3	0.16
II	249.6	151.1	0.21	4	88.8	44.2	2.14	61.0	27.0	0.21
III	331.4	195.2	0.23	8	80.0	57.4	3.13	59.0	26.0	0.28
IV	302.8	221.2	0.24	9	78.0	66.3	4.12	58.0	33.3	0.39

SMA, specific methanogenic activity; BPR, biogas production rate; R-COD, total COD removal efficiency; R-SO_4^{2-}, sulfate removal efficiency; VSS, volatile suspended solids.

removed)$^{-1}$ d^{-1}], which was attributed to the substantial increase (∼50%) of the VSS concentration at RB (Fig. 3), together with the increase of the SMA (0.23 gCOD-CH_4 gVSS^{-1} d^{-1}). Macroscopic granules with a maximum size of 8 mm also were observed at the end of this phase (Table 3).

Phase IV also showed an increase of adaptation for SRB, indicated by an increase in the amount of S produced per unit of sulfate added [221.2 mgS (gSO_4^{2-})$^{-1}$]. As VSS concentration in the sludge remained approximately constant (45.5 gVSS L^{-1}), activity of SRB increased and the CH_4 yield decreased (with respect to Phase III) to 302.8 mL CH_4 (gCOD removed^{-1}) d^{-1}. Maximum granule size of 9 mm was observed at the end of the start-up period (Table 3).

The biogas composition showed stable CH_4 and CO_2 concentrations during the experiment, ranging from 58 to 61%, and from 26 to 33%, respectively. However, H_2S concentration in the gas phase increased gradually from 0.16 to 0.39% from phases I to IV, in agreement with the increased R-SO_4^{2-} and S yield (Table 3).

In general, for the applied operating conditions, the development of macroscopic granules (9 mm maximum size), the R-COD close to 80%, the BPR >4 m^3 m_R^{-3} d^{-1} and the CH_4 concentration in biogas of ≈58%, agreed with indicative values reported for UASB reactors,[4,7,12,18] considered as accurate indicators of completion of the start-up after a short term of 54 days. Additional studies on the granule quality, focusing on the effect of the applied conditions on the sludge settleability, and size distribution of the granules could provide further information for understanding the start-up process of a laboratory-scale UASB reactor treating a sulfate-rich sugar cane vinasse.

CONCLUSIONS

A successful strategy for the start-up operation of a UASB reactor fed with sugar cane vinasse was applied. The results showed that biomass was able to assimilate OLRs from 3 to 10 kgCOD m_R^{-3} d^{-1}, reducing VFAs accumulation in less than five days, while increasing BPR to above 4 m^3 m_R^{-3} d^{-1}. The COD and SO_4^{2-} concentration profiles showed homogenous distribution in the blanket and settler zones, whereas the compounds were removed mainly in the bed zone of the reactor. However, a slight decrease of total and free sulfide concentrations along the reactor height was observed due to the mass transfer of H_2S between the liquid and gas phases, suggesting that the sludge bed in UASB reactors fed with sulfate-rich liquid substrates are exposed to H_2S concentrations higher than those measured in the reactor effluent. During the start-up period (54 days), the development of macroscopic granules, the COD removal efficiency, the BPR and the CH_4 content proved successful indicators to monitor the start-up of the reactor, and their values reached 9 mm, 78%, 4.12 m^3 m_R^{-3} d^{-1} and 58%, respectively. Further research will be needed for strategy validation in pilot and full-scale UASB reactors treating vinasse.

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