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ORIGINAL PAPER

Performance of staged and non-staged up-flow anaerobic sludge bed (USSB and UASB) reactors treating low strength complex wastewater

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Abstract The use of anaerobic processes to treat low-strength wastewater has been increasing in recent years due to their favourable performance-costs balance. For optimal results, it is necessary to identify reactor configurations that are best suited for this kind of application. This paper reports on the comparative study carried out with two high-rate anaerobic reactor systems with the objective of evaluating their performances when used for the treatment of low-strength, complex wastewater. One of the systems is the commonly used up-flow anaerobic sludge blanket (UASB) reactor. The other is the up-flow staged sludge bed (USSB) system in which the reactor was divided longitudinally into 3, 5 and 7 compartments by the use of baffles. The reactors (9 l) were fed with a synthetic, soluble and colloidal waste (chemical oxygen demand (COD) < 1000 mg/l) and operated at 28°C and 24 h hydraulic retention time. Intermediate flow hydraulics, between plug-flow and completely-mixed, in the UASB and 7 stages USSB reactors allowed efficient degradation of substrates with minimum effluent concentrations. Low number of compartments in the USSB reactors increased the levels of short-circuiting thus reducing substrate removal efficiencies. All reactors showed high COD removal efficiencies (93–98%) and thus can be regarded as suitable for the treatment of low strength, complex wastewater. Staged anaerobic reactors can be a good alternative for this kind of application provided they are fitted with a large enough (≥7) number of compartments to fully take advantage of their strengths. Scale factors seem to have influenced importantly on the comparison between one and multi staged sludge-bed reactors and, therefore, observations made here could change at larger reactor volumes.

Keywords Compartmentalised reactor · Dilute wastewater · Phase separation · Reactor hydraulics

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Introduction

The anaerobic technology is a mature and well-established process for biological wastewater treatment. Over the last 30 years, an improved understanding of the microbiology and better reactor designs have enabled the treatment of a wide range of wastewaters, particularly highly concentrated and recalcitrant wastes to which much of the attention has been focused. More recently, anaerobic reactors have also been applied to the removal of organic matter from dilute (<1000 mg chemical oxygen demand (COD)/l) particulate and soluble wastes such as sewage.

The anaerobic treatment of domestic wastewater has been experimented mainly in developing countries where its relatively low costs and acceptable performance make it an attractive option for sanitation (Barbosa and Sant'Anna 1989; Foresti 2001; Wiegant 2001). However, the characteristics of these wastes might pose a number of challenges to anaerobic technologies (Kalogo and Verstraete 1999; Seghezzo et al. 1998), especially to those based on the sludge-bed principle, the most widely applied process. Since organic matter concentration is low and influent flow-rates can vary widely, reactor design is based on hydraulic rather than on organic load and the reactor needs high biomass retention (Lettinga and Hul shoff Pol 1991). Soluble organic matter can be degraded rapidly but suspended solids might pass through the reactor with little degradation thus reducing overall COD removal efficiency. Biomass washout can also be a concern at high influent flow-through. Since significantly smaller amounts of sludge are produced, care must be taken so that biomass washout does not exceed the biomass production inside the reactor. Gas production is also low which results in little agitation and poor biomass-substrate contact thus affecting COD degradation. It should also be noted that at low substrate levels in the bulk of the reactor, reaction rates, according to Monod kinetics, might be just a fraction of those observed when the substrate is not growth limiting (Speece 1996). Apparently due to these reasons and, in some cases, because of scale factors, COD removal efficiencies observed in up-flow anaerobic sludge bed (UASB) reactors have not exceeded 60–85% over a wide range of temperatures (10–28°C) and hydraulic retention times (HRT, 2–48 h) (Behling et al. 1997; Kalogo and Verstraete 2000; Lew et al. 2003; Ruiz et al. 1998; Singh and Viraraghavan 1998).

To overcome some of the problems encountered in UASB reactors, the use of compartmentalised reactor configurations has been suggested. High solids retention and minimum biomass washout is sought by introducing baffles along the reactor length. Compartmentalisation can also promote the separation of the different phases of anaerobic degradation thus providing optimal conditions for the hydrolysis, acidification and methanisation of the retained solids as they pass through the system. The treatment of a dilute soluble and colloidal waste using anaerobic baffled reactors (ABR) with more than 80% COD removal efficiencies (35°C and 6–80 h HRT) has been reported (Langenhoff et al. 2000).

So far only horizontal flow staged reactors have been used for the treatment of dilute complex wastes. Investigations have focused on the evaluation of factors such as temperature, retention time and loading rate (Gopala Krishna et al. 2009; Langenhoff and Stuckey 2000; Langenhoff et al. 2000) but the effect of the number of compartments on reactor performance has not been addressed. In particular, the assessment of UASB and compartmentalised reactors operating under similar conditions has largely been overlooked. This paper reports on the comparative study carried out with a UASB, used as reference, and 3, 5 and 7 stages up-flow sludge bed (USSB) reactors with the objective of evaluating their performance when used for the treatment of a low-strength, complex waste.

Materials and methods

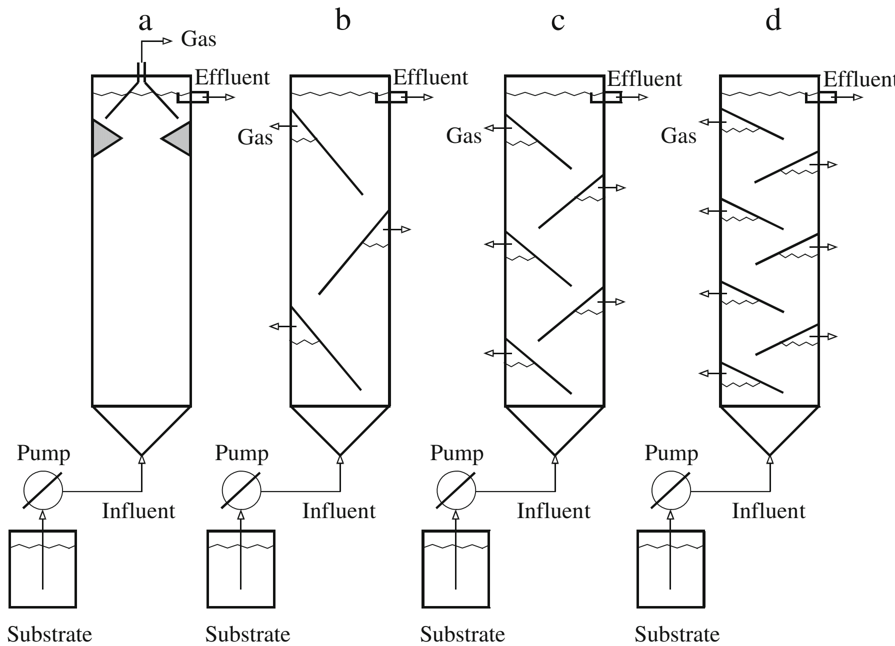
Experimental set-up

Four reactors (9 l each) made of clear acrylic plastic (80 cm height and 12 × 12 cm cross section) were used (Fig. 1). One of the reactors was a conventional UASB whereas the other three were configured as up-flow staged sludge bed reactors with three, five and seven compartments: USSB3, USSB5 and USSB7, respectively. Details on the characteristics of this reactor design can be found elsewhere (van Lier et al. 1994). Influent supply to the reactors was provided intermittently by peristaltic pumps under the control of a timer (3.5 min on, every 30 min). HRT was set to 24 h which resulted in hydraulic and COD loading rates of 0.04 l/l.d and 0.9 g COD/l.d, respectively. Reactors were operated at ambient temperature (28 ± 2°C). Each reactor was seeded with approximately 7.2 l of sludge from a mesophilic pilot scale UASB reactor treating sewage (65 g volatile suspended solids (VSS)/reactor). This was sufficient to fill all compartments.

Wastewater

The reactors were fed with a soluble and colloidal synthetic waste made of whole milk powder (0.5 g/l)

Fig. 1 Experimental set up: **a** UASB, **b** 3 staged USSB, **c** 5 staged USSB and **d** 7 staged USSB



and dry dog food (1.5 g/l) diluted with tap water. The feed was freshly prepared each day. Sodium bicarbonate was added (1 g $NaHCO_3$ per g COD) to maintain suitable buffering such that the system pH could be kept within the range of 6.8–7.2. The general physico-chemical characteristics of the synthetic waste closely resembled those of typical domestic wastewater (Table 1). COD concentration of the feed varied along the study due to heterogeneous dog food blending and dissolving. A similar waste has previously been used with a reported average particle size of >500 µm (Langenhoff et al. 2000). All reactors were fed from the same feed batch and inlet tubing was allowed to reach the bottom of the feeding containers so that all particles were introduced to the reactors.

Analyses

Several parameters were measured in the feed, the effluent and, eventually, in the compartments of the reactors for which liquid and sludge samples were taken through the installed sampling ports.

For the characterisation of the synthetic waste, the following analyses were done in accordance to standard methods (APHA 1995): fifth day carbonaceous biochemical oxygen demand, total nitrogen, total phosphate and oil and grease.

For routinely assessment of reactors performance, total and soluble COD were measured by the closed-reflux colorimetric method (APHA 1995). In the case of the soluble COD test, samples were centrifuged at 13,000 rpm for 10 min before analysis. Total and volatile suspended solids (TSS and VSS) were measured gravimetrically by filtering, drying the residue at 103°C to a constant weight and then igniting it at 550°C (APHA 1995). Turbidity and pH were measured with calibrated lab meters. Alkalinity and total volatile acids were measured by titration with sulphuric acid to pH 5.75 and 4.3 according to the method proposed by Jenkins et al. (1983).

For measurement of volatile fatty acids (VFA: acetic, propionic and butyric acids), samples were centrifuged at 15,000 rpm for 20 min and a 1 ml aliquot from the supernatant was acidified with 10 µl 50% (v/v) HCl. Analysis was then performed in a gas chromatograph fitted with a flame ionisation detector (GC-FID, Perkin Elmer, model Clarus 500). A Nukol (Supelco) capillary column was used (15 m × 0.53 mm × 0.5 µm). Operating temperatures for the injection port and the detector were the same at 250°C. The temperature in the oven started at 100°C and gradually increased to 200°C at a rate of 10°C/min. H_2 was used as carrier gas at 1.5 ml/min. The correlation coefficients (R^2) for the standard calibration curves of these VFA were higher than 0.98 in all cases.

Table 1 Physico-chemical characteristics of the wastewater

Parameter	This study[a]	Typical domestic wastewater[b]
pH	7.5 (0.5)	
Total chemical oxygen demand (mg/l)	859 (233)	250–1000
Soluble chemical oxygen demand (mg/l)	727 (220)	
Biochemical oxygen demand at 5 days (mg/l)	560 (50)	110–400
Total suspended solids (mg/l)	333 (45)	100–350
Volatile suspended solids (mg/l)	267 (30)	80–275
Oil and grease (mg/l)	570 (35)	50–150
Total nitrogen (mg/l)	43 (5)	20–85
Total phosphorous (mg/l)	8 (2)	4–15
Total alkalinity (mg/l as $CaCO_3$)	710 (85)	50–200

[a] Standard deviation in brackets

[b] Metcalf and Eddy Inc (1991)

Hydrodynamic flow characteristics

Residence time distribution (RTD) studies were carried out to analyse the hydrodynamics of each reactor by adding a pulse of inert tracer to the inlet (5 g NaCl in 50 ml water). Samples were taken from the outlet of the reactors, at regular intervals of 1 h, for at least 3 HRTs after the addition of the pulse and were analysed for NaCl concentration by using a conductivity meter. Conductivity was correlated to saline concentration by linear calibration, and the conductivity was corrected for the background level in the feed. Tests were conducted in duplicate in each reactor. The recovery of the tracer was more than 90% in all experiments. With the collected data, the exit age distribution function (E), mean retention time and variance were calculated and the dispersion and tanks-in-series models were fitted (Levenpiel 1999).

Examination of biomass

The structural characteristics of the microbial aggregates were studied using a scanning electron microscope (SEM, Topcon, model 510) at 10–15 kV acceleration. Samples were first washed for 5 min with distilled water in a 5 ml vial and then fixed for 30 min with glutaraldehyde at 3% in 0.1 M (pH = 7) phosphate buffer. The samples were washed again for 5 min with 0.1 M (pH = 7) phosphate buffer and next were dehydrated with 30, 70, 90 and 100% ethanol, 1:1 (v/v) acetone-ethanol and 100% acetone, each for 15 min. Finally, the samples were dried to the CO_2 critical point in a drier (Spi Supplies, model SPI-Dry CPD), mounted on an aluminium cylinder with conductive carbon tape and covered with gold–palladium in a conductive metal depositer (Denton Vaccum, model Desk II).

Methanogenic activity bioassays

The acetoclastic methanogenic activity of the sludge in the reactors was measured by batch bioassays. The tests were performed in 120 ml serum bottles filled with 40 ml of a phosphate buffer (0.1 M Na and K) spiked with sodium acetate (3 g COD/l). The medium contained (per litre): 2.07 g of NH_4Cl, 0.674 g of K_2HPO_4, 0.248 g of $(NH_4)_2SO_4$, 0.12 g of $NaHCO_3$, 3 ml of trace element solution (containing per litre: 500 mg of EDTA, 2000 mg of $FeCl_2.4H_2O$, 100 mg of $NiCl_2.6H_2O$, 150 mg of $CoCl_2.6H_2O$, 50 mg of $(NH_4)_6Mo_7O_{24}.4H_2O$, 50 mg of $ZnCl_2$, 40 mg of $CuCl_2.2H_2O$, 30 mg of $AlCl_3$, 500 mg of $MnCl_2.4H_2O$) and 0.4 ml of Na_2S-cistein solution (containing per litre: 8 g of NaOH, 12.5 g of cistein HCl, 12.5 g of $Na_2S{\cdot}9H_2O$). The gas phase used in the bottles was N_2. The bottles were inoculated with 3 g VSS of the sludge used to start-up the reactors or that obtained from the reactors at the end of the run. Incubation temperature was 35°C. Microbial activity of the bioassays was calculated by measuring the daily uptake of soluble COD per gram of VSS biomass added to the bottle. The experiments were performed in triplicate and controls were run with all samples.

Statistical analysis

20 successive data points for each reactor (effluent COD, turbidity and volatile acids) were selected from the whole data set obtained near the end of the run (steady state) and were statistically analysed. For

turbidity and volatile acids, a completely randomised block design was used whereas for COD the design applied was a completely randomised unbalanced block. In both cases, the block was the running time and the factor the number of compartments. Tukey's test at 95% confidence was used to compare the treatments. All analyses were performed on the software Statistica 6.1.

Results and discussion

Reactors performance

Figures 2, 3, 4, and 5 show the performance of the reactors during the study. Effluent COD progressively decreased with time in all reactors (Figs. 2b, 3b, 4b, 5b) until very low and stable COD concentrations were attained by the end of the run. Correspondingly, COD removal efficiency improved along the study from acceptable to very good levels in all reactors (Figs. 2e, 3e, 4e, 5e). Mean values under stable operation varied from 90% total COD and 93% soluble COD removal by the USSB3 reactor to 98% -both CODs- removal by the USSB7 reactor.

A turbidity meter was not available at the beginning of the study and measurements started from day 78 (Figs. 2c, 3c, 4c, 5c). Turbidity reflects the solids content of a sample and hence the levels of this parameter were largely reduced by all reactors as a result of solids entrapping and degradation in the sludge bed. Mean influent turbidity value was 89 NTU (based on the whole data set) and the reactors brought this down to less than 15 NTU.

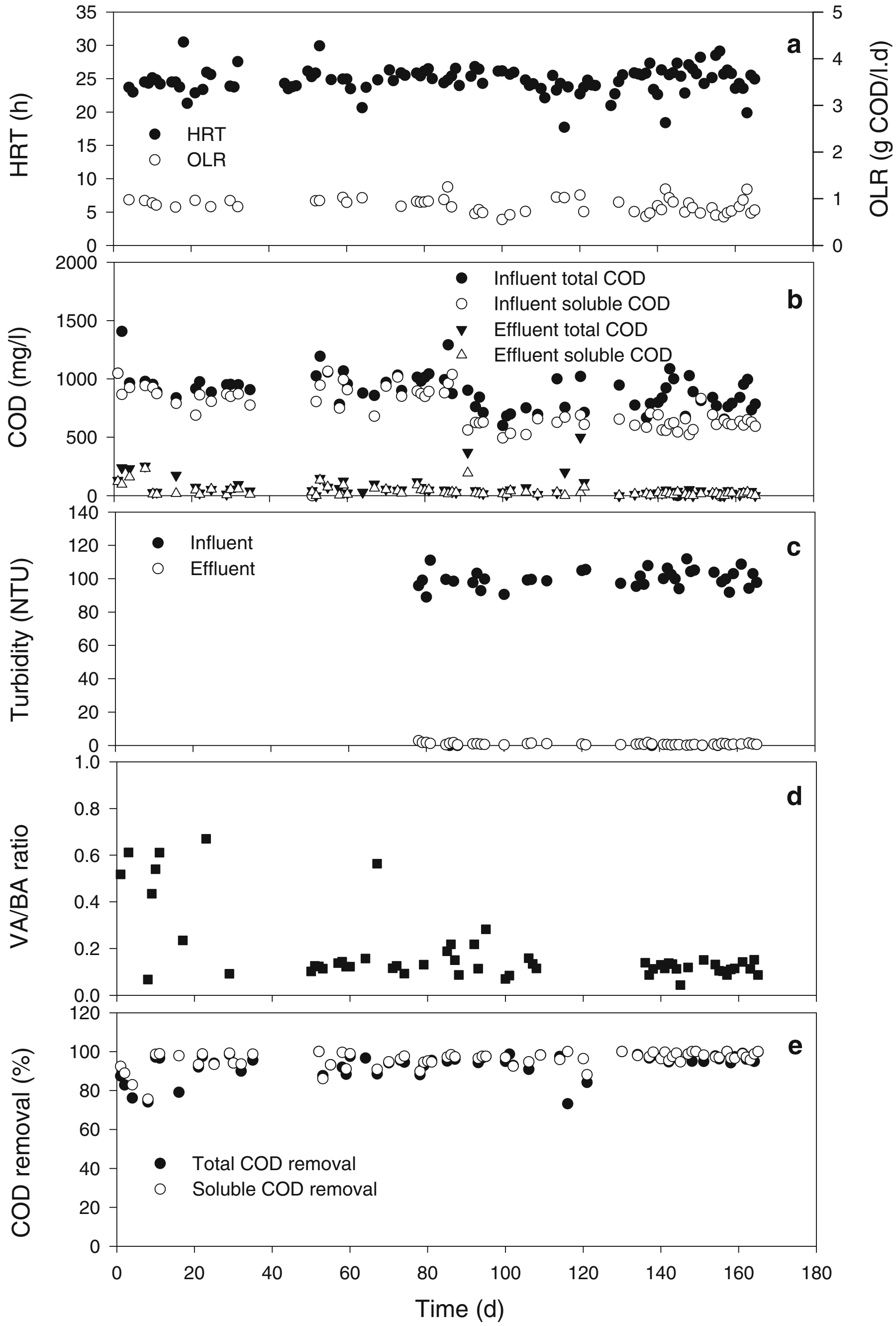
The stability of the reactors can be inferred from the volatile acids to bicarbonate alkalinity (VA/BA) ratio shown in Figs. 2d, 3d, 4d, 5d. During the start-up period, VA/BA ratio values as high as 0.8 were recorded. According to the scale proposed by Switzenbaum et al. (1990), VA/BA ratio values between 0.4 and 0.8 indicate some instability in the reactor. As the methanogenic activity increased with time, volatile acids were degraded more rapidly and the VA/BA ratio fell to a mean value of 0.1 in all reactors indicating stable operation.

In order to evaluate whether the performance of the reactors was significantly different from each other, a data set of 20 single effluent measurements for COD, turbidity and volatile acids, obtained under stable conditions, was statistically analysed. Results are shown in Table 2. Total COD in the effluent of the USSB reactors decreased with the increase in the number of compartments and all reactors showed significantly different levels of removal. However, the UASB reactor shared characteristics with the USSB5 and the USSB7 and was second best. On the other hand, all reactors, except the USSB3 that showed the highest mean value, achieved similarly low soluble CODs in the effluents and were not significantly different from each other. Similarly to total COD, all reactors showed significantly different levels of turbidity in the effluents and the values of this parameter decreased with the increase in the number of compartments except for the UASB which showed the lowest level of turbidity. Effluent volatile acids too showed the same trend of change with the number of compartments and, as COD, the minimum level of volatile acids was observed in the USSB7 reactor. Here it should be noted that although the easy and quick method used for routine monitoring overestimated VFA values, the results are still useful for comparative purposes.

Flow characteristics

Tracer studies were conducted to investigate whether hydrodynamic flow characteristics could help explain the observed trends in reactor performance. The results of the RTD data analysis are shown in Table 3. As expected, it was found that the dispersion number (D/μl) decreased and the number of theoretical tanks-in-series (N) increased as the number of compartments increased in the USSB reactors. This trend shows that USSB reactors approach plug-flow conditions as the number of baffles along the vessel length increases. However, even the USSB reactor with the highest number of compartments showed a relatively high dispersion number (0.13) and an N value (4.4) lower than the actual number of compartments in the vessel (7). These results do not necessarily mean that the reactors were well mixed but rather that by-passing and stagnant zones might have occurred, particularly in the USSB3. Axial dispersion might have also been important at long retention times thus contributing to the deviation from the ideal plug-flow model. To confirm this, tests were conducted at the end of the study using non-active reactors (no sludge), tap water as fluid and a pulse of a dye to visualise the flow

Fig. 2 Performance of the UASB reactor: **a** hydraulic retention time (HRT) and organic loading rate (OLR), **b** influent and effluent, total and soluble, chemical oxygen demand (COD), **c** influent and effluent turbidity, **d** volatile acids to bicarbonate alkalinity ratio, **e** total and soluble COD removal



patterns. It was observed that a considerable level of channelling occurred in the USSB3 reactor (data not shown) due to the formation of high velocity profiles in the slots were compartments were interconnected. Large eddies were also observed in the zones below the baffles were hydraulic dead space could have been formed. As a result of this, portions of the dye reached the reactor outlet short after the injection of the tracer. This observation agrees with the mean residence time calculated from the RTD curves in the USSB3 which was considerably shorter (17.4 h) than the theoretical value (24 h). Plug-flow behaviour was approached and flow patterns improved (less short-circuiting and dead zones) with the increase on the number of stages and the reduction of the compartments volume and this impacted positively on the removal efficiencies shown

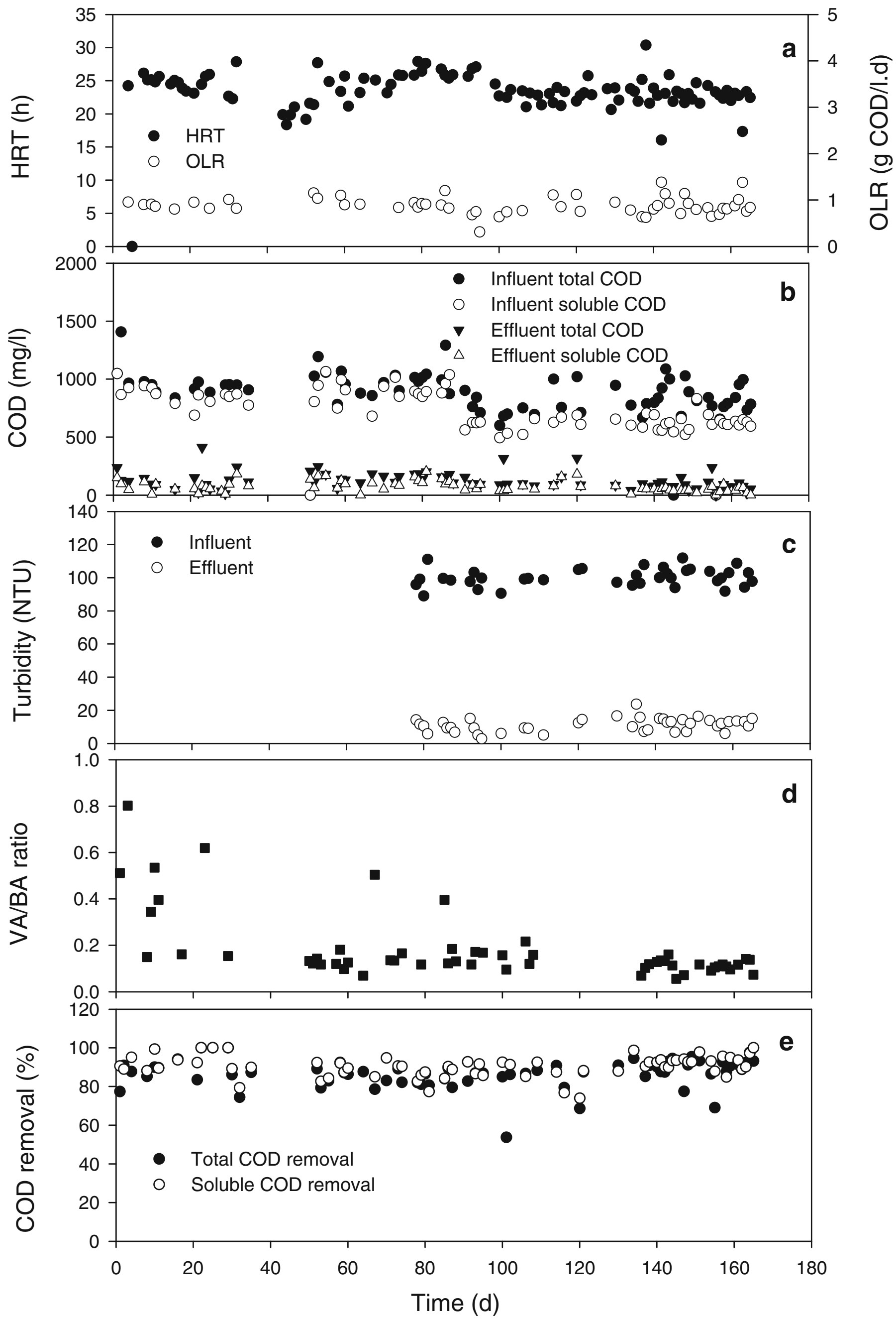
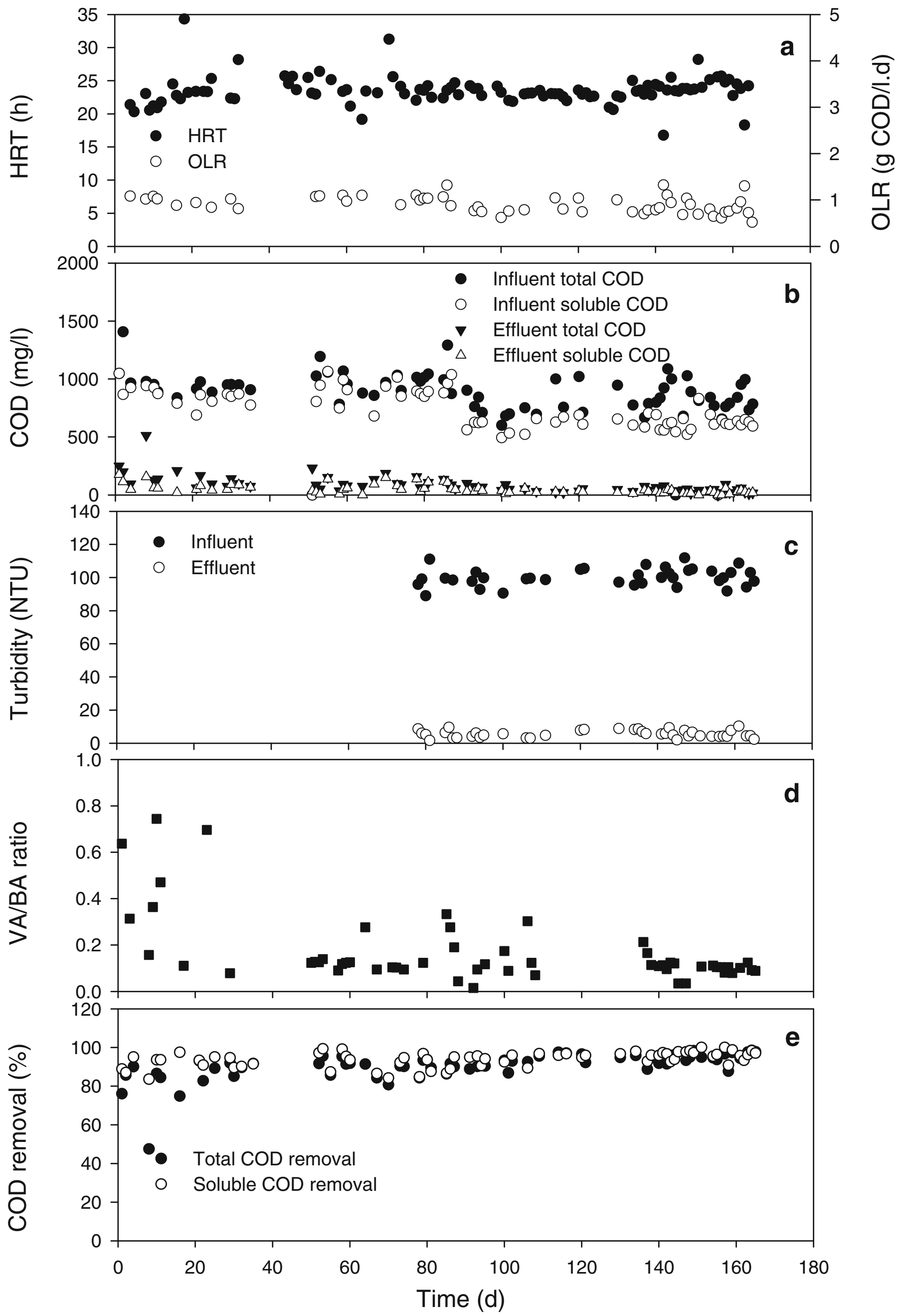


Fig. 3 Performance of the 3 staged USSB reactor: **a** hydraulic retention time (HRT) and organic loading rate (OLR), **b** influent and effluent, total and soluble, chemical oxygen demand (COD), **c** influent and effluent turbidity, **d** volatile acids to bicarbonate alkalinity ratio, **e** total and soluble COD removal

by the USSB5 and, particularly, the USSB7 reactors (Figs. 2, 3, 4, 5 and Table 2).

In a previous study (Lens et al. 1998), a larger value of *N* (9.8) was reported for a USSB reactor with similar characteristics to those used here (5.1 l and 5 compartments) but operated at a much shorter HRT (1.8 h) which might have allowed the formation of streamline flow conditions that more closely resemble plug-flow behaviour. However, even under these conditions the calculated dead zone was large (33%). Similarly, intermediate levels of dispersion (0.054 and 0.046), large values of *N* (9 and 11) and important dead zones (18.5 and 25.4%) were estimated in an 8 chamber ABR (10 l) at shorter HRTs of 8 and 10 h, respectively (Gopala Krishna et al. 2009).

Fig. 4 Performance of the 5 staged USSB reactor: **a** hydraulic retention time (HRT) and organic loading rate (OLR), **b** influent and effluent, total and soluble, chemical oxygen demand (COD), **c** influent and effluent turbidity, **d** volatile acids to bicarbonate alkalinity ratio, **e** total and soluble COD removal



The UASB showed a dispersion number (0.16) and *N* value (3.8) comparable to those of the USSB7 reactor (Table 3). However, the RTD curve showed a longer tail which resulted in an estimated mean residence time (31 h) much larger than the theoretical value (24 h). This suggests that an important amount of hydraulic—although not necessarily biological—dead space occurred in the UASB reactor. Intermediate values of *N* (3.14–3.31) and large dead zones (40.7–48.2%) were also reported by Lens et al. (1998) in a 6.5 l UASB reactor even thought effluent recycling was used to improve mixing. In this study, mixing was poor due to low loading and biogas production rates. However, gentle movement in the sludge bed was sporadically observed in the UASB reactor due to sludge buoyancy followed by settlement as the entrapped gas bubbles

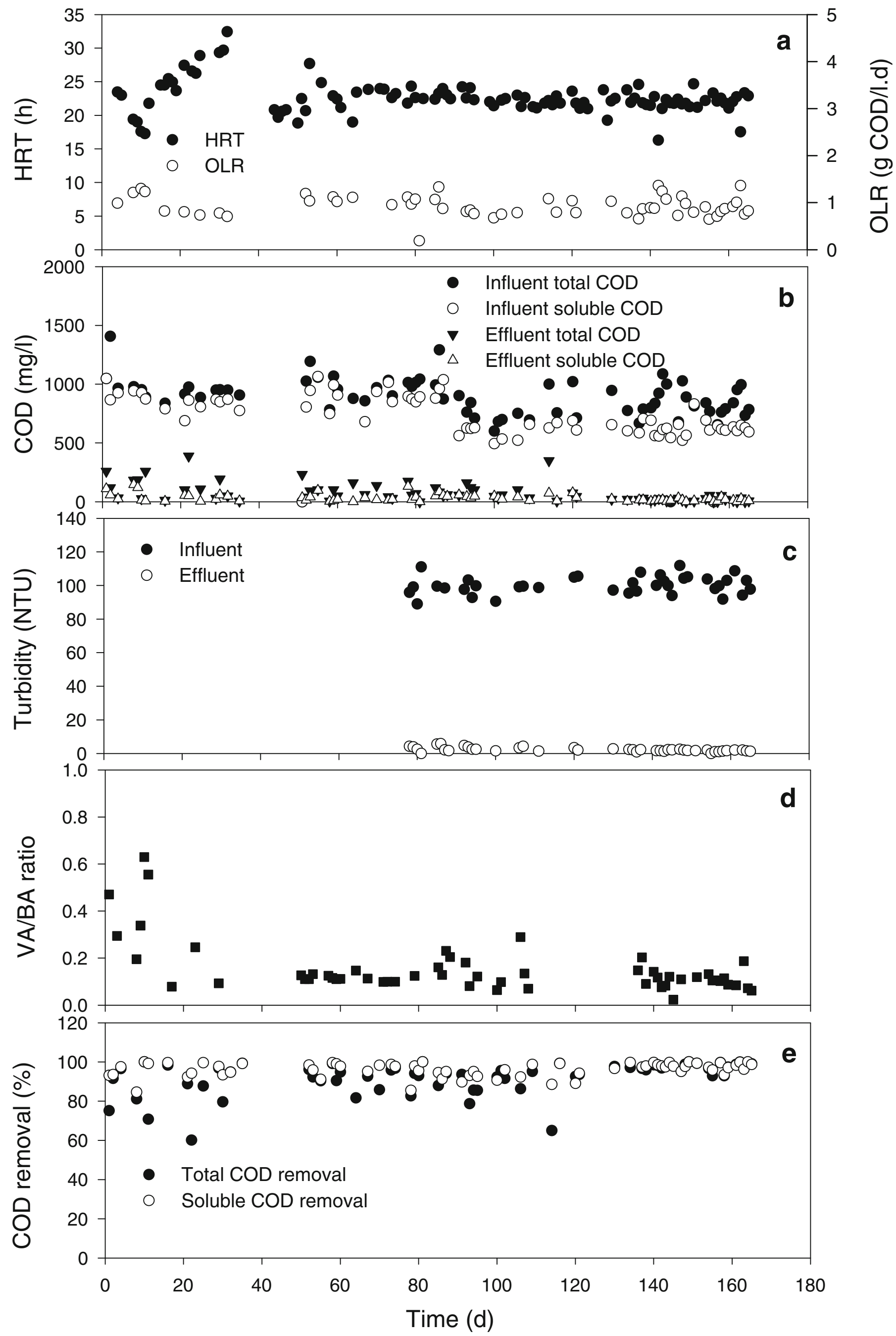


Fig. 5 Performance of the 7 staged USSB reactor: **a** hydraulic retention time (HRT) and organic loading rate (OLR), **b** influent and effluent, total and soluble, chemical oxygen demand (COD), **c** influent and effluent turbidity, **d** volatile acids to bicarbonate alkalinity ratio, **e** total and soluble COD removal

were released. This exchange of materials seems to have allowed biological activity to be maintained in the entire sludge bed, even in the hydraulic stagnant zones where substrates were retained and effectively degraded thus counterbalancing the negative effects of channelling in the sludge bed.

All together the results obtained here indicate that, under the conditions tested, the flow patterns within all reactors were intermediate between plug-flow and perfectly mixed. Any deviation from ideal behaviour could have been caused by channelling, dead spaces and axial dispersion due to the long HRT. Mixed flow

Table 2 Statistical comparison of reactors performance assessed by effluent concentrations

Parameter	Reactor	Mean value (mg/l)	Comparison[a]	d.f.	m.s.	*F*	*P*-value
Total COD	USSB 3 stages	78	A	3	76.998	32.814	5.88E−12
	USSB 5 stages	42	B				
	UASB	28	BC				
	USSB 7 stages	20	C				
Soluble COD	USSB 3 stages	40	A	3	27.815	17.781	1.51E−7
	USSB 5 stages	26	B				
	UASB	23	B				
	USSB 7 stages	16	B				
Turbidity	USSB 3 stages	11.8	A	3	14.028	227.94	1.25E−30
	USSB 5 stages	5.3	B				
	USSB 7 stages	1.6	C				
	UASB	0.5	D				
Volatile acids	USSB 3 stages	95	A	3	2217.8	12.09	5.14E−6
	USSB 5 stages	84	AB				
	UASB	74	BC				
	USSB 7 stages	70	C				

[a] Tukey's test at 95% confidence

hydraulics in the UASB and USSB7 reactor allowed the efficient degradation of substrates with minimum effluent concentrations. Low number of compartments in the USSB5 and, particularly, in the USSB3 reactors increased the levels of short-circuiting thus reducing substrate removal efficiencies. The trend observed in reactor performance can be explained by reactors hydraulics since removal efficiency and N correlated well (correlation coefficient = 0.979). Higher values of N led to better removal efficiencies (Table 3). This is to be expected since a plug-flow reactor will more effectively remove substrate than a completely-mixed reactor with Monod or first-order biological kinetics (Batstone et al. 2005).

As already mentioned, the UASB reactor did not show good mixing conditions due to low loading rates, low biogas production, reactor geometry and small scale. Similarly, Batstone et al. (2005) demonstrated that a laboratory-scale UASB reactor had plug-flow hydraulics whilst a full-scale UASB reactor had mixed flow hydraulics more closely approaching completely-mixed conditions. Therefore, it should be borne in mind that scale might have played an important roll in the comparison between the UASB and USSB reactors in this study and the observations made here could probably change as scale increases.

Table 3 Results of the residence time distribution studies

Reactor	Mean residence time (h)	D/μl	*N*	Average COD removal (%)
UASB	31.0	0.16	3.8	97
USSB3	17.4	1.22	1.3	93
USSB5	20.3	0.20	3.1	95
USSB7	20.1	0.13	4.4	98

COD and VFA profiles

In order to identify the patterns followed during organic matter degradation, samples were withdrawn at different reactor heights and soluble COD and volatile acids profiles were obtained. Figure 6 shows the soluble COD profiles for the four reactors. As expected, COD decreased progressively as water flowed through the sludge bed in the different compartments of the reactors and reached the outlet port. However, differences can be observed between reactors. COD started high near the inlet port of the USSB3 reactor and then decreased linearly along the reactor height. Around 120 mg soluble COD/l still

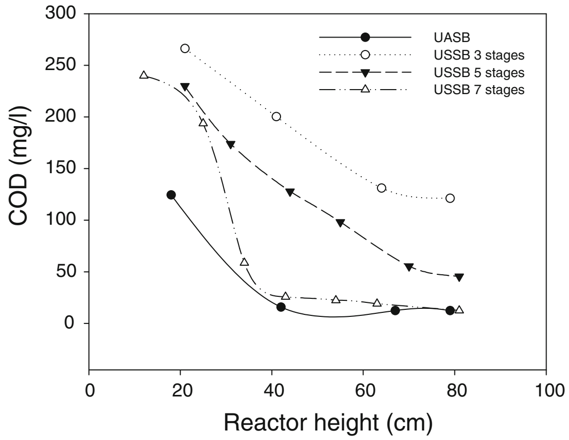


Fig. 6 Evolution of soluble COD concentration over reactors height on operational day 160

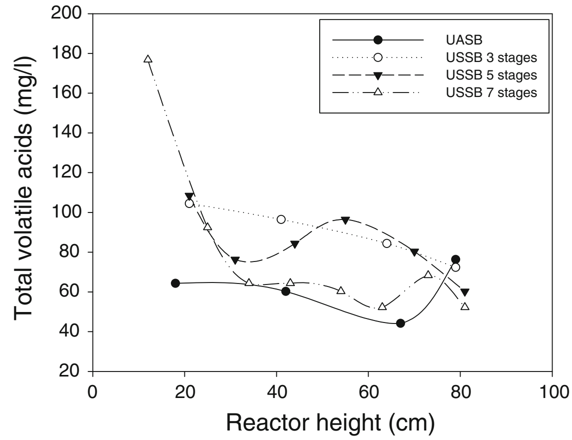


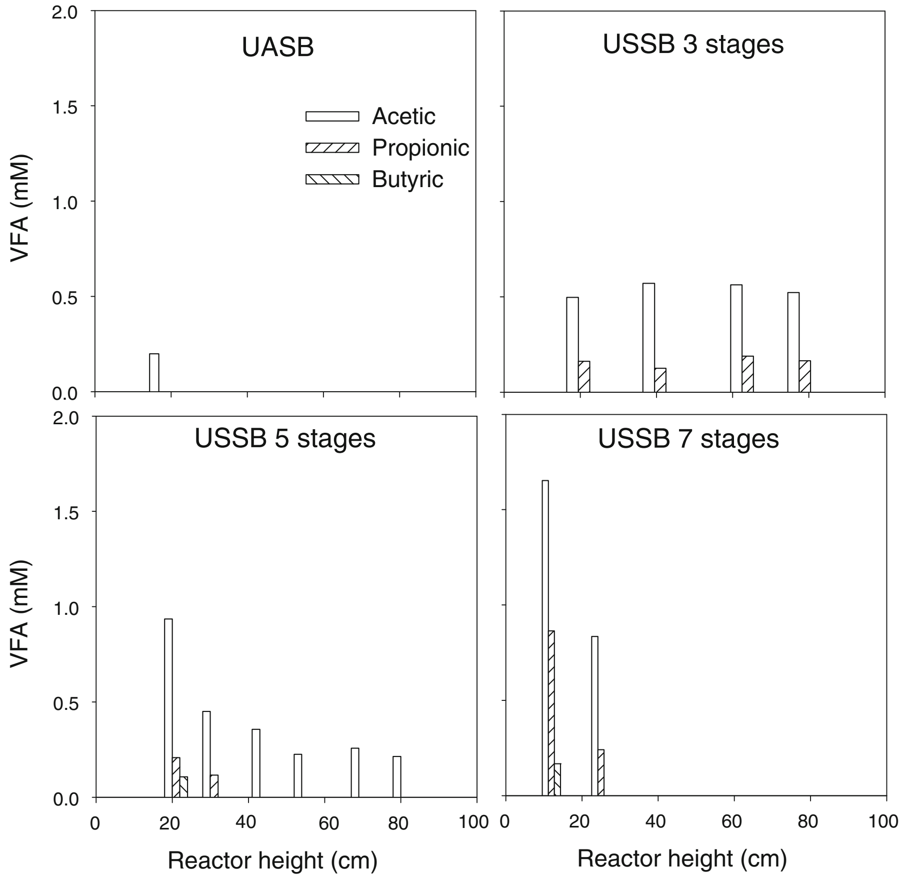
Fig. 7 Evolution of total volatile acid concentration over reactors height on operational day 160

remained in the USSB3 effluent. The COD pattern observed in the USSB5 is about the same as that of the USSB3, except that effluent COD concentration was lower than in the USSB3. In the USSB7, COD near the inlet port was similar to the values observed in the USSB3 and USSB5 reactors. Here however COD felt sharply in the next 30 cm and kept decreasing, although at a much lower rate, until a low value of 12 mg COD/l was reached in the effluent. Soluble COD near the inlet port of the UASB reactor was the lowest of all and, similarly to the USSB7, it decreased, initially at a fast rate then slowly, until a value of 12 mg COD/l was attained. The higher COD levels at the bottom of the USSB reactors can be explained by the higher COD loading that accumulates in the lowest compartments due to their smaller volumes. In the UASB, influent COD load was probably diluted by the reactor contents.

A similar pattern can also be seen from the volatile acids (VA) profiles along the height of each reactor (Fig. 7). The highest VA level was found at the bottom of the USSB7 due probably to the accumulation of the organic load in its compartments with the lowest volume. This high load might have encouraged high acidogenic and low methanogenic activities thus resulting in the accumulation of volatile acids. Conversely, the level of volatile acids at the bottom of the UASB was the smallest of all reactors at that location due probably to dilution but also to a higher methanogenic activity.

In order to identify the volatile fatty acids present at different heights in the reactors, samples were withdrawn and then analysed by gas chromatography. Figure 8 shows the VFA patterns found in each reactor. In general, the tendency observed in VFA patterns is qualitatively in agreement with those of VA shown in Fig. 7. However, quantitatively some discrepancies were found. As mentioned earlier, these differences are most probably due to the inaccuracy of the titration method used for VA measurement where dissolved CO_2 could have resulted in false positive VA values. VFA concentration near the inlet port of the UASB was the lowest of all values found at that location in all reactors and consisted solely of acetic acid. Acetic acid was then utilised in the upper sections of the UASB. In the USSB3, acetic acid at the bottom was higher than that found in the UASB at the same location and propionic acid was also found. Both, acetic and propionic acids, remained at similar concentrations in the rest of the USSB3 reactor and its effluent. In the USSB5, an even higher level of acetic acid was found in the lowest section of the reactor and here propionic and butyric acids were also present. However, unlike the USSB3, in the USSB5 propionic and butyric acids were completely consumed in the upper sections of the reactor and acetic acid was greatly reduced. Following this tendency, acetic, propionic and butyric acids found in the lowest section of the USSB7 were the highest found in all reactors. However, in this reactor VFA were quickly consumed upwards and the effluent was free of any of them. These results suggest that acidogenic and methanogenic activities co-occurred along the sludge bed in the UASB reactor whereas in

Fig. 8 Volatile fatty acids composition and distribution along each reactor height on operational day 160



the USSB reactors, acidogenic and methanogenic activities were segregated as the number of compartments increased. In the USSB7 reactor, in particular, a high acidogenic activity was observed at the bottom whilst methanogenesis occurred mainly at higher levels. A similar trend was reported in a USSB with 5 compartments (Lens et al. 1998). In the USSB3 reactor, hydraulic anomalies such as bypassing might have led to the washout of VFA produced at the bottom to the upper sections of the reactor where methanogenic activity was poorer. In general, COD and VFA profiles (Figs. 6, 7, and 8) are consistent with reactor performance data described previously since they show that both the UASB and USSB7 reactors produced the best effluent quality.

Similarly to what was observed here, the COD removal efficiency shown by a granular bed baffled reactor (GRABBR) was only slightly higher than that of a UASB (Shanmugam and Akunna 2008). The GRABBR showed greater process stability at low HRT whilst the UASB was seen to be better equipped to cope with overloads or shockloads due to its higher dilution capacity.

The high COD removal efficiencies achieved by the four reactors studied here (93–98%) compare well with data reported in the literature. For example, the treatment of complex, dilute (≤ 500 mg COD/l), synthetic wastewater in baffled reactors has resulted in COD removals of 85–92.5% at 20–24 h HRT (Gopala Krishna et al. 2008; Langenhoff et al. 2000; Manariotis and Grigoropoulus 2002). Similarly, the anaerobic treatment of a low strength (500 mg COD/l) synthetic wastewater in a 140 l UASB reactor under ambient temperature (20–35°C) and a short HRT (3 h) resulted in 90–92% COD reductions (Singh et al. 1996).

Sludge characteristics

In order to find out if the differences observed in reactor performances could be related to biomass characteristics, samples of sludge were withdrawn

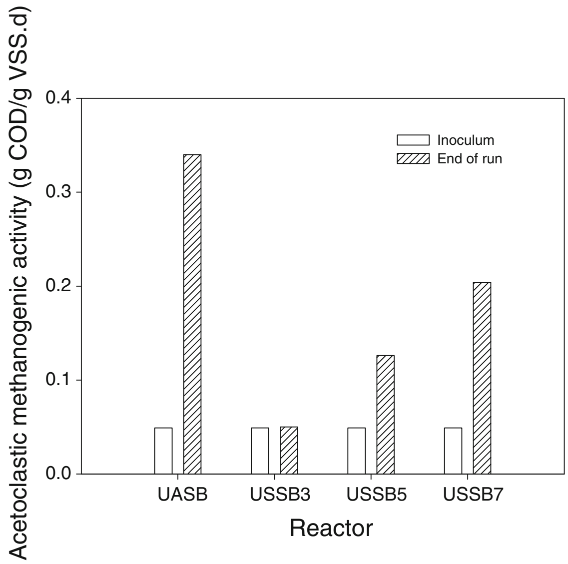


Fig. 9 Acetoclastic methanogenic activity of the sludge in each reactor at the start and at the end of the study

from around half the height of each reactor and their acetoclastic methanogenic activity (AMA) was then measured. Figure 9 compares the AMA of the sludge samples at the start and at the end of the run. Methanogenic activity in the inoculum was low but after reactors operation it importantly increased in all reactors except the USSB3. AMA increased with the number of compartments in the USSB reactors but the sludge from the UASB showed the highest value of all. The tendency shown by sludge AMA is consistent with the information gathered from reactors performance and COD/VFA profiles. The higher methanogenic activities observed in the UASB and USSB7 could explain the superior performance of these two reactors. These higher methanogenic activities might have been favoured by the better hydraulic conditions found in these two reactors.

Figure 10 shows the morphological characteristics of sludge from the UASB and USSB5 reactors. The sludge from the three USSB reactors showed similar architectural characteristics. Morphological observations led to the identification of important differences between the sludge grown in the UASB and in the USSB reactors. In the UASB, sludge consisted mainly of dark, small and compact granules (1–2 mm diameter) whereas in the USSB reactors biomass was found to grow in the form of greyish, slimy and large aggregates (>5 mm). These aggregates collapsed more easily during handling than the granules from the UASB. Upon SEM examination, the surface of the UASB granules was seen to be covered by a film—maybe extracellular polysaccharides (EPS)—which kept the granules compact. Also, the surface of the UASB was densely packed whilst in the USSB aggregates large cavities were observed. Filamentous, coccal, spiral and rod shaped bacteria proliferated in both granules and aggregates. However, bamboo-shaped rods, possibly *Methanosaeta*, also densely covered and were more evident in the USSB aggregates.

The above observations agree with a previous report (Uemura et al. 2005) that formation of granular type sludge was not possible in a three staged UASB reactor and the sludge retained persisted as large flocs. Shanmugam and Akunna (2008) also reported the formation of greyish and slimy bacterial aggregates in a granular bed baffled reactor (10 l, 5 compartments). Similarly, different bacterial morphologies were observed in an ABR (Gopala Krishna et al. 2009). The greatest variety of hydrolysing and acid producing microorganisms was found in the inlet compartment whilst the acetoclastic methanogen *Methanosaeta* was most prevalent upstream at lower acetate concentrations. This is to be expected as the bacterial population observed in anaerobic sludge is related to the concentration and type of the available substrates. Phase separation within the reactors was concluded from morphological observations (Gopala Krishna et al. 2009; Shanmugam and Akunna 2008).

Conclusions

Up-flow staged sludge bed reactors have been shown here to be a good alternative for the anaerobic treatment of complex low-strength wastewater because: (i) plug-flow reactor hydraulics are approached which more effectively remove substrate than a completely-mixed reactor under Monod or first-order biological kinetics, especially at low substrate concentrations, (ii) solids are effectively entrapped and removed in the sludge bed and (iii) phase separation occurs promoting the efficient degradation of substrates.

COD removal efficiency increased (up to 98%) with the number of compartments. A low number of compartments (3) increased the levels of short-circuiting thus reducing substrate removal efficiencies.

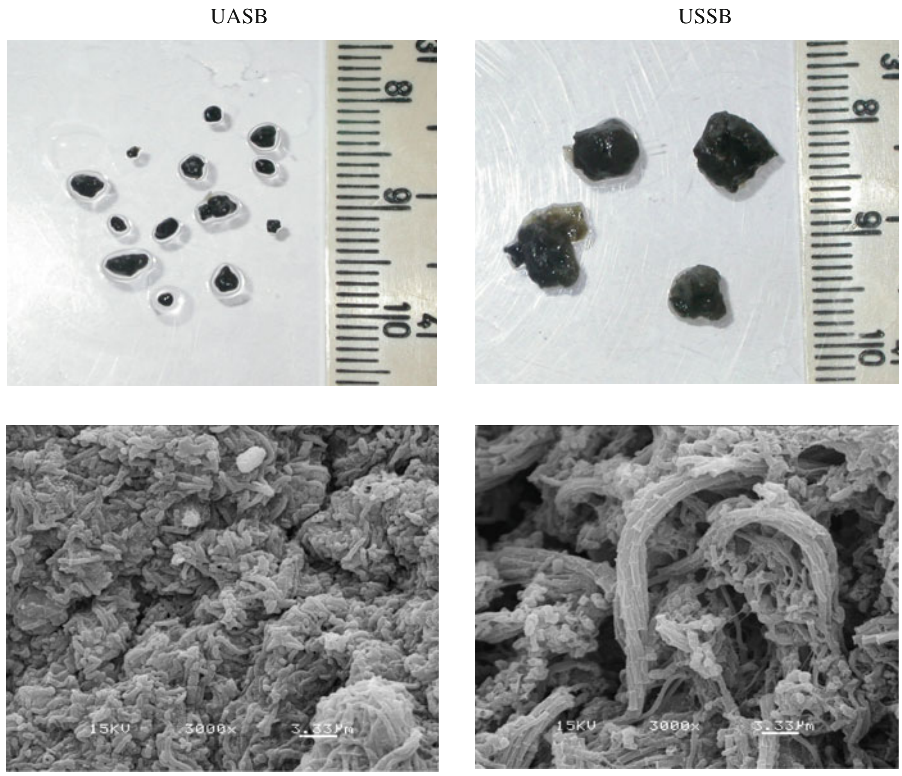


Fig. 10 Morphological characteristics of the biological sludge retained in the UASB and USSB reactors

Therefore, reactors should be fitted with a large enough (~7) number of compartments to fully take advantage of their strengths.

Hydrodynamic flow patterns influenced biomass characteristics and microbial activities which explained reactors performance. Flow hydraulics in the UASB and 7 stages USSB reactors were similar, intermediate between plug-flow and perfectly mixed with deviation from ideal behaviour due to by-passing and dead spaces, which resulted in comparable reactor performance. The UASB reactor did not show good mixing conditions due to low loading rates, low biogas production, reactor geometry and small scale but this might change at an increased scale. Therefore, the observations made here during the comparison of the UASB and USSB reactors could change at larger reactor volumes.

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References

APHA (1995) Standard methods for the examination of water and wastewater, 19th edn. American Public Health Association, Washington, DC

Barbosa RA, Sant'Anna G Jr (1989) Treatment of raw domestic wastewater in a UASB reactor. Water Res 23:1483–1490

Batstone DJ, Hernandez JLA, Schmidt JE (2005) Hydraulics of laboratory and full-scale upflow anaerobic sludge blanket (UASB) reactors. Biotechnol Bioeng 91:387–391

Behling E, Díaz A, Colina G, Herrera M, Gutiérrez E, Chacin E, Fernández N, Forster CF (1997) Domestic wastewater treatment using a UASB reactor. Bioresour Technol 61:239–245

Foresti E (2001) Perspectives on anaerobic treatment in developing countries. Water Sci Technol 44:141–148

Gopala Krishna GVT, Kumar P, Kumar P (2008) Treatment of low strength complex wastewater using an anaerobic baffled reactor (ABR). Bioresour Technol 99:8193–8200

Gopala Krishna GVT, Kumar P, Kumar P (2009) Treatment of low-strength soluble wastewater using an anaerobic baffled reactor (ABR). J Environ Manage 90:166–176

Jenkins SR, Morgan JM, Sawyer CL (1983) Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration. J Water Pollut Control Fed 55:448–453

Kalogo Y, Verstraete W (1999) Development of anaerobic sludge bed (ASB) reactor technologies for domestic wastewater treatment: motives and perspectives. World J Microbiol Biotechnol 15:523–534

Kalogo Y, Verstraete W (2000) Technical feasibility of the treatment of domestic wastewater by a CEPS-UASB system. Environ Technol 21:55–65

Langenhoff AAM, Stuckey DC (2000) Treatment of dilute wastewater using an anaerobic baffled reactor: effect of low temperature. Water Res 34:3867–3875

Langenhoff AAM, Intrachandra N, Stuckey DC (2000) Treatment of dilute soluble and colloidal wastewater using an anaerobic baffled reactor: influence of hydraulic retention time. Water Res 34:1307–1317

Lens PNL, van den Bosch MC, Hulshoff Pol LW, Lettinga G (1998) Effect of staging on volatile fatty acid degradation in a sulfidogenic granular sludge reactor. Water Res 32:1178–1192

Lettinga G, Hul shoff Pol W (1991) UASB process design for various types of wastewaters. Water Sci Technol 24: 87–107

Levenpiel O (1999) Chemical reaction engineering. Wiley, New York

Lew B, Belavski M, Admon S, Tarre S, Gree M (2003) Temperature effect on UASB reactor operation for domestic wastewater treatment in temperate climate regions. Water Sci Technol 48:25–30

Manariotis ID, Grigoropoulus SG (2002) Low-strength wastewater treatment using an anaerobic baffled reactor. Water Environ Res 74:170–176

Metcalf and Eddy Inc (1991) Wastewater enginering, treatment and reuse. Tchobanoglous G, Burton FL, Stensen HD (rev.). McGraw-Hill, New York

Ruiz I, Soto M, Veiga MC, Ligero P, Vega A, Blázquez R (1998) Performance of and biomass characterisation in a UASB reactor treating domestic waste water at ambient temperature. Water SA 24:215–222

Seghezzo L, Zeeman G, van Lier JB, Hamelers HVM, Lettinga G (1998) A review: the anaerobic treatment of sewage in UASB and EGSB reactors. Bioresour Technol 65: 175–190

Shanmugam AS, Akunna JC (2008) Comparing the performance of UASB and GRABBR treating low strength wastewaters. Water Sci Technol 58:225–232

Singh KS, Viraraghavan T (1998) Start-up and operation of UASB reactors at 20°C for municipal wastewater treatment. J Ferm Bioeng 85:609–614

Singh KS, Harada H, Viraraghavan T (1996) Low-strength wastewater treatment by a UASB reactor. Bioresour Technol 55:187–194

Speece RE (1996) Anaerobic biotechnology for industrial wastewater. Archae Press, Nashville, TN

Switzenbaum MS, Giraldo-Gómez E, Hickey RF (1990) Monitoring of the anaerobic methane fermentation process. Enzyme Microb Technol 12:722–730

Uemura S, Harada H, Ohashi A, Torimura S (2005) Treatment of wastewater containing a large amount of suspended solids by a novel multi-staged UASB reactor. Environ Technol 26:1355–1362

van Lier JB, Boersma F, Debets MMWH, Lettinga G (1994) High rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. Water Sci Technol 30:251–261

Wiegant WM (2001) Experiences and potential of anaerobic wastewater treatment in tropical regions. Water Sci Technol 44e:107–113