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USE OF A GRIFFITH TUBE TO EVALUATE THE ANAEROBIC SLUDGE SEDIMENTATION IN A UASB REACTOR TREATING AN EFFLUENT WITH LONG-CHAIN FATTY ACIDS

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Abstract - This paper proposes to study the sedimentation characteristics of anaerobic sludge, by determining the settling velocity of sludge granules with the Griffith Tube. This is a simple, low-cost method, suitable for use in full-scale treatment plants. The settling characteristics of sludge from two laboratory-scale UASB reactors fed with saccharose and different concentrations of sodium oleate and sodium stereate were evaluated. Addition of fatty acids caused a gradual destabilization of the system, affecting overall performance. The sedimentation profile changed after addition of fatty acids to the synthetic substrate, decreased sedimentation velocity and increased granule diameter. This behaviour was attributed to the adsorption of fatty acids onto the granules, modifying the diameter, shape and density of these bioparticles.
Keywords: Anaerobic process; UASB reactor; Long chain fatty acids; Griffith tube; Sludge settleability.

INTRODUCTION

Anaerobic wastewater treatment biotechnology has been greatly advanced by the development of the upflow sludge bed reactor concept, such as the upflow anaerobic sludge bed (UASB) (Kim *et al*., 2001). The success of anaerobic systems is related to their ability to accumulate good settling biomass without the need for a biomass carrier, allowing high solids retention time and process stability with simple and low-cost equipment (Ahn and Speece, 2003), high organic carbon removal and a small footprint (Batstone *et al*., 2004).

Bacterial aggregation, known as granulation, is one of the important operational factors in anaerobic treatment systems (Yan and Tay, 1997; Britz *et al*., 2002). These systems generate granules that are dense conglomerates of microorganisms, microbially produced components such as extracellular polymeric substances and other material from the wastewater influent. Granulation results from microbial auto-immobilisation and, subsequently, aggregate formation and growth. It is essentially a microbial selection pressure that is imposed on the sludge. Several factors may hinder the granulation process during the anaerobic treatment of diluted wastewaters where municipal or domestic wastewater is involved, even when the wastes are pre-hydrolysed, and thus further research is required (Ligero and Soto, 2002). A well-developed granular sludge with high physical

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strength and settling velocity is necessary for optimal operation of UASB reactors (Hickey *et al*., 1991). Benefits include high liquid flow and gas upflow rates together with high settling rates (Lettinga and Hulshoff Pol, 1991) and decreased fines generation due to higher strength compared to flocs (Pereboom, 1997).

Numerous studies report the physical, chemical and biological characteristics and factors that affect development, maintenance and settling of granules during start-up in anaerobic sludge bed reactors (Yu *et al*., 2013; Batstone *et al*., 2004; Li and Yuan, 2002; Quarmby and Forster, 1995; Andras *et al*., 1989; Hamoda and van den Berg, 1984), comparing granules from full-scale reactors with similar wastewaters (Bhatti *et al*., 1995), or examining specific properties such as strength (Pereboom, 1997). The temperature and salt concentration dependent density and viscosity changes of water have great impact on the settling velocity of granular sludge, and can be an important reason for the reported troublesome start-up of granular sludge reactors (Winkler *et al*., 2012).

Oleate (C18:1), stearate (C18:0) and palmitate (C16:0) are the major long chain fatty acids (LCFA) constituents in lipid-rich wastes and wastewaters (Battimelli *et al*., 2010; Valladão *et al*., 2011). Palatsi *et al*. (2012) evaluated the impact of LCFA adsorption on the methanogenic activity in batch assays for two anaerobic granular sludges in the presence and absence of bentonite as synthetic adsorbent. A clear inhibitory effect was observed at an oleate concentration of 0.5 $g.L^{-1}$. According to Zonta *et al*. (2013), the cell-membrane seems to be the prime common target for most of the LCFA inhibitory effects on anaerobic biomass described. According to Kim and Gadd (2008), cell-membrane exposure to high concentrations of LCFA promotes macromolecular crowding and disruption of mechanisms such as protonmotive-force, DNA-docking and ATP-chemosynthesis. Impairment of nutrient uptake or inhibition of specific enzyme activity were also reported (Desbois and Smith, 2010).

According to Thaveesri *et al*. (1995), the LCFA have an amphiphilic structure composed of a hydrophobic aliphatic tail and a hydrophilic carboxylic head. In reactors operating with a pH higher than 7, the sludge granules may disintegrate, since the LCFA begin to act as surfactants, lowering the surface tension. In this case, the adhesion of hydrophilic cells appeared to be enhanced at a low liquid surface tension, while the adhesion of hydrophobic cells was favoured at a high surface tension. Daffonchio *et al*. (1995) observed changes in the hydrophobic characteristics of the biomass in anaerobic reactors when subjected to the presence of surfactants, showing that under operating conditions with low surface tension it may cause a sloughing-off from granular sludge and the selective washout of these microorganisms.

Direct granular particle size analysis has been performed manually with a graticule ruler or by wet sieving (Laguna *et al*., 1999), by automatic image analysis and computerised data processing (Dudley *et al*., 1993; Ahn and Speece, 2003) and by particle size analysis using laser (Yan and Tay, 1997). Indirect granular sludge density analysis has been performed by measuring settling velocity and extrapolation of the corresponding granule diameters (Grothenhus *et al*., 1991) or sludge volume index (Andras *et al*., 1989; Ahn, 2000). Studies on UASB reactors have shown that sludge settling may affect the rate of start-up and maximum reactor loading rate (Hamoda and van den Berg, 1984).

The Griffith tube (Hairsine and McTainsh, 1986) is an adaptation of the siltometer and is designed to provide accurate information on the settling velocity distribution of sediment outside the size range covered by Stoke's Law. It provides information on settling in clear water, without the interactions which could occur in natural systems (Hairsine and McTainsh, 1986). The majority of the sediment from soil, as well as anaerobic sludge granules, commonly exists in the form of aggregates which settle at a rate dependent upon the aggregate's size, shape, roughness and density relative to the fluid. However, Childs (1969) in his review of the assumptions behind Stoke's Law suggests that significant surface and form drag effects limit the use of Stoke's Law to particles of less than 60 μm diameter, while for sediment greater than 60 μm the relationship between size and settling rate is complex and depends on the particular variations found in aggregate shape and density (Gibbs *et al*, 1971).

In anaerobic reactors treating effluents with high LCFA contents, small sludge granules may undergo an aggregation process which is potentiated by LCFA adsorption on the surface of the granules, so that sludge flocs appear and therefore Stoke's law is not applicable.

The settling velocity depends not only on the size, shape and density of the particles and the density and viscosity of the fluid, but also on the number or concentration of the particles (Cheng, 1997b; Li and Yuan, 2002).

Considering the focus on settleability of anaerobic sludge, the main objective of this project was to introduce an assessment protocol for determining the sedimentation characteristics of granular sludge using a Griffith Tube, and to evaluate its applicability

as an operational parameter in laboratory-scale UASB reactors. This protocol was easy to apply, allowing visual observation of the sludge granules during sedimentation, separation and particle size distribution according to the settling time in Griffith Tube. This method also allowed separate analysis of the granules collected in each fraction according to the observed settling time, allowing several evaluations to be performed regarding the characteristics of the biomass.

MATERIALS AND METHODS

UASB Reactors

Two UASB reactors (17.0 L), designated UASB 1 and 2, were set up and were used as the laboratory reactors. The reactors were inoculated with anaerobic sludge obtained from a UASB reactor treating the effluent from a gelatin factory. The sludge was characterized with respect to total volatile solids (TVS), volatile fatty acids (VFA): acetic, n-butyric, i-butyric, valeric and i-valeric acid according to the 5560D method (APHA, 2012). Specific methanogenic activity (SMA) was determined according to Monteggia (1991) and Miranda *et al*. (2005), using sodium acetate as substrate. The hydraulic retention time (HRT) was 24 h at all periods. The operational conditions of the UASB reactors are shown in Table 1, where it can be observed that the affluent organic load rate increased in the UASB 1 reactor to 4.16, 4.86 and 6.26 kg COD m^3 d^{-1} with the addition of sodium oleate to the saccharose substrate during the periods of operation II (94 days), III (57 days) and IV (28 days), respectively. The same can be observed for the UASB 2 reactors, with an affluent organic load rate of 4.19, 4.92 and 6.38 kg COD.m^{-3}.d^{-1} by adding sodium stearate to the saccharose substrate during the same operational periods.

The sludge inoculum was adapted for 230 days to a synthetic wastewater containing saccharose (3.1 g.L^{-1}), sodium bicarbonate (3.1 g.L^{-1}), ammonium chloride (0.6 g.L^{-1}), trisodium phosphate (0.12 g.L^{-1}) and yeast extract (0.091 g.L^{-1}), equivalent to a chemical oxygen demand (COD) of 3.450 mg.L^{-1}. Operating temperature was 35.0 ± 0.5 ºC.

Settleability Assessment Protocol

The sedimentation of the sludge granules was measured using the Griffith tube. Measurements taken in the inoculum and the sludge from each reactor at the end of periods I, II, III and IV are summarized in Tables 2 and 3.

Settling Test Apparatus

Sedimentation was measured in the Griffith tube, which determines settling velocity in clean water. It was measured at the end of each operational period. The equipment is similar to that used by Hairsine and McTainsh (1986), and is an adaptation of the Siltometer. The Griffith tube, as shown in Figure 1, has three basic components: the settling tube, the sample introduction device and the collection turntable on which settled samples are collected.

The Sample Introduction Device

The sample input chamber is in the upper part of the tube and is composed of an acrylic chamber containing a stainless steel cup into which the sludge is placed. The cup is held in a metal device that allows it to be rotated from without, allowing the sample to fall into the sedimentation tube. This chamber has a conical acrylic lid that allows it to be completely sealed. The lower part of the lid is glued to the sedimentation tube. At the back of the sample introduction device there is an exit (Figure 1G) linked to a vacuum pump that allows the water to be transferred from the turntable to the upper part of the sedimentation tube, maintaining the water column pressure at -2 m.

Table 1: Operational conditions of UASB reactors, according to the added substrate.

Operation Period	I	II	III	IV
Operation Time (d)	**230**	**94**	**57**	**28**
UASB 1	**Substrate: Saccharose**	**Substrate: Saccharose + Sodium oleate**		
Concentration (mg.L^{-1})	3090	250	500	1000
$^{b}COD_t$ (mg.L^{-1})	3450	4192	4914	6359
OLR (kg COD.m^{-3}.d^{-1})	3.45	4.16	4.86	6.26
UASB 2	**Substrate: Saccharose**	**Substrate: Saccharose + Sodium stereate**		
Concentration (mg.L^{-1})	3090	250	500	1000
$^{b}COD_t$ (mg.L^{-1})	3450	4201	4932	6394
OLR (kg COD.m^{-3}.d^{-1})	3.45	4.19	4.92	6.38

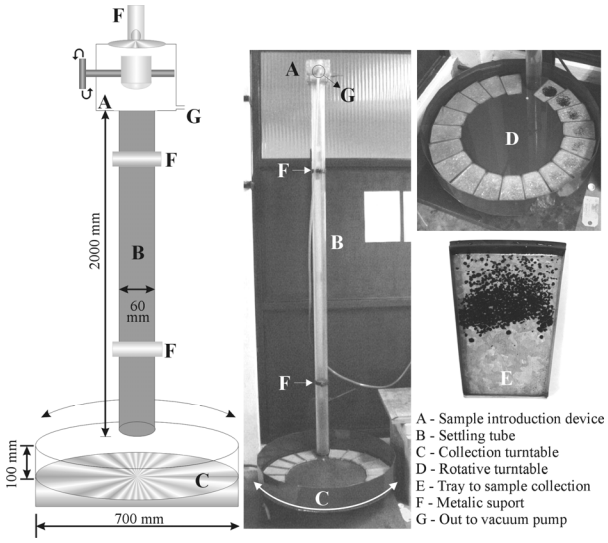

Figure 1: Griffith tube. Adapted from Hairsine and McTainsh (1986).

The Settling Tube

The semi-fixed acrylic tube is 2000 mm in length and 60 mm in internal diameter, and is held by 3 arms in an aluminium track. This can be advanced a few centimetres vertically, thus moving the lower portion of the sedimentation tube towards or away from the collection point.

The Sample Collection Turntable

The sample collection system consists of a rotating circular table, like a turntable in the form of a tray, 700 mm in external diameter and 100 mm deep. The base of the turntable is coupled to a central axis attached to a metal base, allowing the system (turntable and trays for sample collection - Figure 1, detail "D" and "E") to turn on its own axis. In Figure 1D, the 19 sample receiving points (Figure 1E) are seen inside the largest tray (turntable). Each of these 19 trays corresponds to a sub-sample; these are submersed in water at the base of the tray. Each receiving point (Figure 1E) is 10 mm in depth and the tapered shape shown in Figure 1D allows them to fit closely together such that all settling granules are collected. Water in the collection tray seals the tube to prevent air from entering and displacing the hanging column of water in the settling tube.

Griffith Tube - Principle of Operation

The method is based on maintaining a water column inside the tube under a negative pressure of approximately 2 m. The sludge sample is placed in the cup in the sample introduction device. The lever is turned, releasing the sample into the tube and beginning the sedimentation process. The first fraction of the sample is collected in the first collecting tray (Figure 1D-E). After a given interval, the turntable is moved and positioned in the lower part of the sedimentation tube. This is repeated up to the 19th tray. The time during which the bottom of the sedimentation tube remains over each tray is determined in advance using a sludge sample which is released into the sedimentation tube and allowed to deposit completely within a single tray. The time between the beginning of this process, and when the last particles settle, is divided by the number of trays (Figure 1E), giving the time interval during which the bottom of the sedimentation tube should remain over each tray. Thus 19 samples will be collected for equal time intervals. At the end of the collection, 10 min is allowed with the point of the tube over the final tray to ensure the collection of the final few particles.

Particle Size Analysis

The mean diameter of the sludge granules collected in each tray of the sample collection turntable was determined with a laser particle size analyzer (CILAS 1180) with a range of 0.04 μm to 2.500 μm in 100 divisions.

Analytical Methods

Samples of effluent were collected twice a week and COD, alkalinity and pH were measured according to APHA (2012). The SMA tests were analysed according to Monteggia (1991) and Miranda *et al*. (2005). The SMA determinations involved samples collected at the first sampling point from the base of the reactor, on days 0 and 230 (Period I) and at the end of each period (II, III and VI). The gas composition and the concentrations of volatile fatty acids (VFA) were analysed by gas chromatography. For VFA a Varian 3700 gas chromatograph was used, equipped with a flame ionization detector (FID) at 160 ºC, injector at 170 ºC and a 6 ft x 4 mm diameter Chromosorb 100/120 W/AW (Supelco Inc. USA) column (15% SP1220/1% H_3PO_4). The helium carrier gas flow was 50 ml min^{-1} and the oven temperature program was 80 ºC for 3.0 min^{-1}, with a 9 ºC min^{-1} ramp to 120 ºC and the final hold at 180 ºC for 5 min. The level of total solids (TS), volatile solids (VS) and fixed solids (FS) was analysed according to the 2540A method (APHA, 2012) for each subsample of the sedimentation test.

RESULTS AND DISCUSSION

Characterization of the Inoculum

The VS concentration was 62.53 $g.L^{-1}$ and the SMA was 0.0066g COD_{CH4}.g $VS^{-1}.d^{-1}$. The SMA test showed that the sludge had a good activity, with more than 80% methane in the biogas. The sedimentation characteristics of inoculum sludge granules are shown in Table 2, where it can be observed that about 81.7% of the mass of the particles sedimented within the first 2 min, with settling velocities between 0.83 and 1.67 $cm.s^{-1}$. The particles were considered spheres and had a mean diameter of 1212.1 to 1358.6 μm (Figure 2A).

This frequency distribution indicates that the sludge is homogeneous (unimodal profile), with good sedimentation properties. In general, sludges with

Table 2: Sedimentation characteristics of UASB sludge inoculum using Griffith Tube.

Sub-sample	Mass (%)	VS (%)	Mean diameter (μm)	Settling (min)	Settling velocity ($cm.s^{-1}$)
Inoculum					
1	68.70	49.7	1358.6	2	1.67
2	13.00	11.1	1212.1	4	0.83
3	3.00	2.6	636.5	6	0.55
4 to 19	15.30	1.5 – 0.03	752.4 - 212.2	6 to 50	0.42 to 0.09

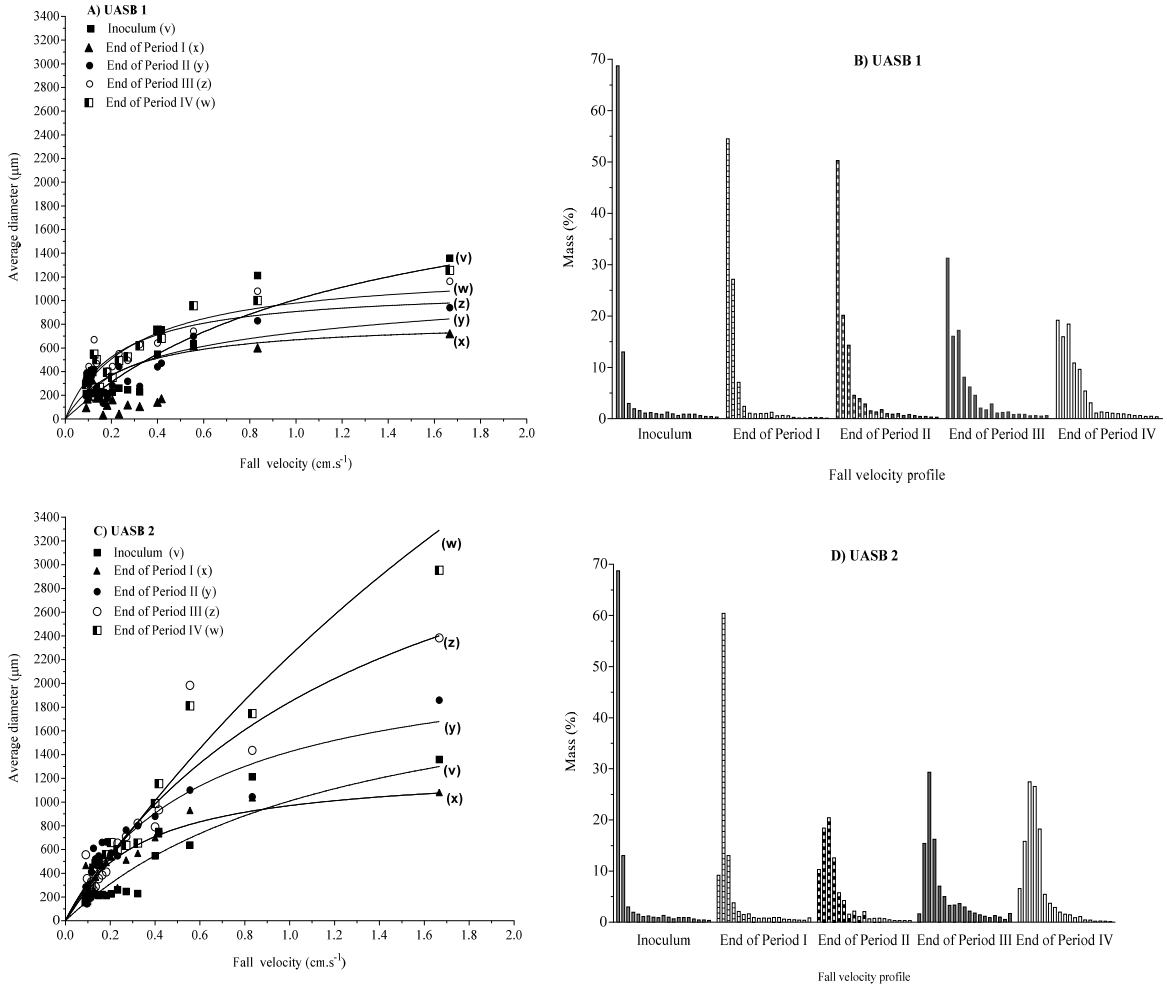

Figure 2: Sedimentation characteristics of the sludge and mean diameter versus settling velocity from UASB 1 and 2 collected in the Griffith tube, in each period studied.

high ST_{50} sediment slowly. The sludge settling characteristics influence the rate of start-up and maximum reactor loading rate. The UASB process performance is limited by the ability of the settler to retain sludge in the system in order to attain a high solids retention time at a low hydraulic retention time. Settling velocity is a critical factor that regulates biosolids-liquid separation and effluent quality in UASB reactors. The changes in the permeability of the sludge granules, due to LCFA adsorption may also have affected the characteristics of sludge sedimentation. At the end of period I, there was a change in the sedimentation profile of the sludge granules. The sludge in UASB 1 retained a homogeneous sedimentation (Figure 2B and Table 3), with more than 80% of the granule mass settling between 0-4 min. This behaviour is taken to be unimodal, with a defined sedimentation profile. UASB 2 showed a similar behaviour at the end of the same period, with 73.4% of the granule mass settling within 2-6 min (Table 3), and the greater fraction settling between 0-4 min, increasing the TS_{50} with respect to the inoculum (Figure 3). This was also seen at the end of period III in UASB 2, while UASB 1 only showed a decrease in percent mass for those granules that sedimented between 0-6 min, leading to an increase in mass of the granules that sedimented at lower velocities than the inoculum (Table 3 and Figure 2).

Table 3: Summary of sedimentation test results from UASB reactors using a Griffith Tube.

Sub-sample	Mass (%)	VS (%)	Mean diameter (μm)	Settling (min)	Settling velocity ($cm.s^{-1}$)
UASB 1 – End of Period I					
1	54.80	92.5	2700.0	2	1.67
2	27.30	46.2	2200.0	4	0.83
3	7.10	12.1	618.0	6	0.55
4 to 19	10.80	3.7 – 0.05	330.4 – 171.4	6 to 50	0.42 to 0.09
UASB 1 – End of Period II					
1	46.80	93.1	940.0	2	1.67
2	18.70	51.4	830.0	4	0.83
3	13.20	21.3	698.0	6	0.55
4 to 19	21.00	65.4-98.5	471.0 - 132.0	6 to 50	0.42 to 0.09
UASB 1 – End of Period III					
1	0.00	0.0	1358.6	2	1.67
2	23.41	94.8	1212.1	4	0.83
3	25.06	94.1	636.5	6	0.55
4 to 19	51.60	67.9-99.0	744.4 – 319.0	6 to 50	0.42 to 0.09
UASB 1 – End of Period IV					
1	20.64	2.5	1255.0	2	1.67
2	17.26	96.4	1000.4	4	0.83
3	19.86	97.2	956.4	6	0.55
4 to 19	42.24	74.8 - 99.1	750.4 – 290.5	6 to 50	0.42 to 0.09
UASB 2 – End of Period I					
1	9.20	8.6	2220	2	1.67
2	60.40	56.2	2000	4	0.83
3	13.00	12.1	587.7	6	0.55
4 to 19	17.40	3.3 – 0.2	493.9 – 36.2	6 to 50	0.42 to 0.09
UASB 2 – End of Period II					
1	12.40	14.3	1858.2	2	1.67
2	22.09	98.8	1436.2	4	0.83
3	24.56	97.3	1983.4	6	0.55
4 to 19	25.83	77.5 - 99.2	933.9 – 210.3	6 to 50	0.42 to 0.09
UASB 2 – End of Period III					
1	0.0	0.0	2383.2	2	1.67
2	15.60	97.4	1036.2	4	0.83
3	29.80	96.4	983.4	6	0.55
4 to 19	54.60	78.3 - 96.4	733.9 – 235.6	6 to 50	0.42 to 0.09
UASB 2 – End of Period IV					
1	5.72	0.1	2954.2	2	1.67
2	13.72	98.5	1745,5	4	0.83
3	23.83	99.1	1810.2	6	0.55
4 to 19	56.73	66.4 - 99.3	1155.3 – 160.5	6 to 50	0.42 to 0.09

Settling Characteristics

The sedimentation profile of the sludge granules is shown in Figure 2 where the sedimentation velocity ($cm.s^{-1}$) of granules in each sub-sample is plotted versus mean diameter (μm) (Fig. 2A and C) and profile of sedimentation of the sludge collected in each sub-sample in the Griffith tube (mass (%) x sedimentation velocity ($cm.s^{-1}$)) (Fig. 2B and D).

The sedimentation test results for the inoculum and samples from the UASB reactors at each interval analysed are summarized in Table 3. The aliquots collected in each of the 19 trays (sub-sample) were grouped based on the sedimented mass of sludge and the particle diameter. Trays 1, 2 and 3 received the largest sludge mass percentage, culminating in greater settling velocity compared to the other sub-samples, except during periods III and IV, when the addition of LCFA changed the profile of sludge settleability.

The difference between the sedimentation profile of the granules in UASB 1 and 2 at the end of period I may reflect the inhomogeneous nature of the sludge collected at the site before inoculating the reactors; a large volume was collected in order to avoid rupturing the granules and to reduce contact with oxygen.

There was also an increase in mean diameter of the granules after the addition of LCFA (Table 3 and Figure 2A and 2C); this was not accompanied by an increase in sedimentation velocity.

At the end of periods I and III several changes were noted. For granules with a settling velocity less than 0.8 $cm.s^{-1}$ there was a reversal in relation to diameter. In this case granules with a greater diameter sedimented at velocities lower than those in the inoculum and than those granules sedimenting at 0.8 and 1.67 $cm.s^{-1}$. This behaviour was seen in all reactors and probably occurred because of alterations in the shape and density of the granules, since the mean granule diameter increased after LCFA addition. According to Rinzema *et al*., (1993), LCFA are adsorbed onto the granules inhibiting the release of biogas from their interior. A similar effect was reported by Hwu, (1997), Petruy (1999) and Alves *et al*., (2001), who studied the biodegradation of different LCFA.

According to Pietsch *et al*. (2003), biological activity has a crucial influence on settling velocity because the rising bubbles hinder the downward movement of the sludge particles. Hence, lower gas production rates mean higher settling rates. It was found that anaerobic sludge particles contain gas bubbles, and the compressibility of the bubbles which are entrapped in the sludge agglomerates was mathematically described; pressure dependent sedimentation characteristics of the sludge particles were found.

Much experimental and analytical information on the settling velocity of a single particle or biogranule is available to guide practical applications, though the problem is far from being completely solved. However, the case most frequently encountered in analyses and predictions on sediment or bio-granule transport is one where more than a solitary particle falls through a fluid. The presence of the other granules will modify the settling velocity of an individual granule in the fluid, due to mutual interference among granules. For example, a few closely spaced particles in a fluid will fall faster than a single particle. On the other hand, the settling velocity of particles uniformly dispersed throughout a fluid will be less than that of an identical isolated particle in a clear fluid (Cheng, 1997b).

Figure 3A shows biogas production during period I, where the gradual increase of biogas production was seen as the sludge adapted to the substrate. Figure 3B shows the evolution of the concentration of inflowing and outflowing COD concentration, with increased addition of sodium stereate and sodium oleate to the reactors, while Figures 3C and D show relations between organic loads applied in each period in the reactors, and the variation in the concentration of methane in biogas during the experimental period. The decrease of biogas production and methane concentration clearly reflects the influence of the increased LCFA concentration on the influent.

UASB Reactors 1 and 2 – Period I – Substrate: Saccharose

The UASB reactors were started up with an OLR of 3.45 kg COD $m^{-3}.d^{-1}$, using saccharose as the only substrate during period I. COD removal over this time increased from 38% for UASB 1 and 63% for UASB 2 on the second day of operation, to 88% and 91% respectively, at the end of period I. Alkalinity and pH were stable throughout the period. SMA was decreased from 0.0066 (inoculum) to 0.0040g $COD_{CH4}.g^{-1}$ $VS.d^{-1}$ in the reactors at the end of period I. Total VFA decreased from 1011.7 $mg.L^{-1}$ for UASB 1 and 1004.4 $mg.L^{-1}$ for UASB 2 on day 2 of operation, to 73.2 and 85.7 $mg.L^{-1}$ respectively, at the end of period I. The inoculum sludge was removed from a UASB reactor treating effluent from a gelatine factory. Therefore, the high VFA values observed at the beginning of period I are residuals resulting from the original conditions of the reactors where the inoculum was obtained. The VFA values at the end of Period I show that the biomass was well

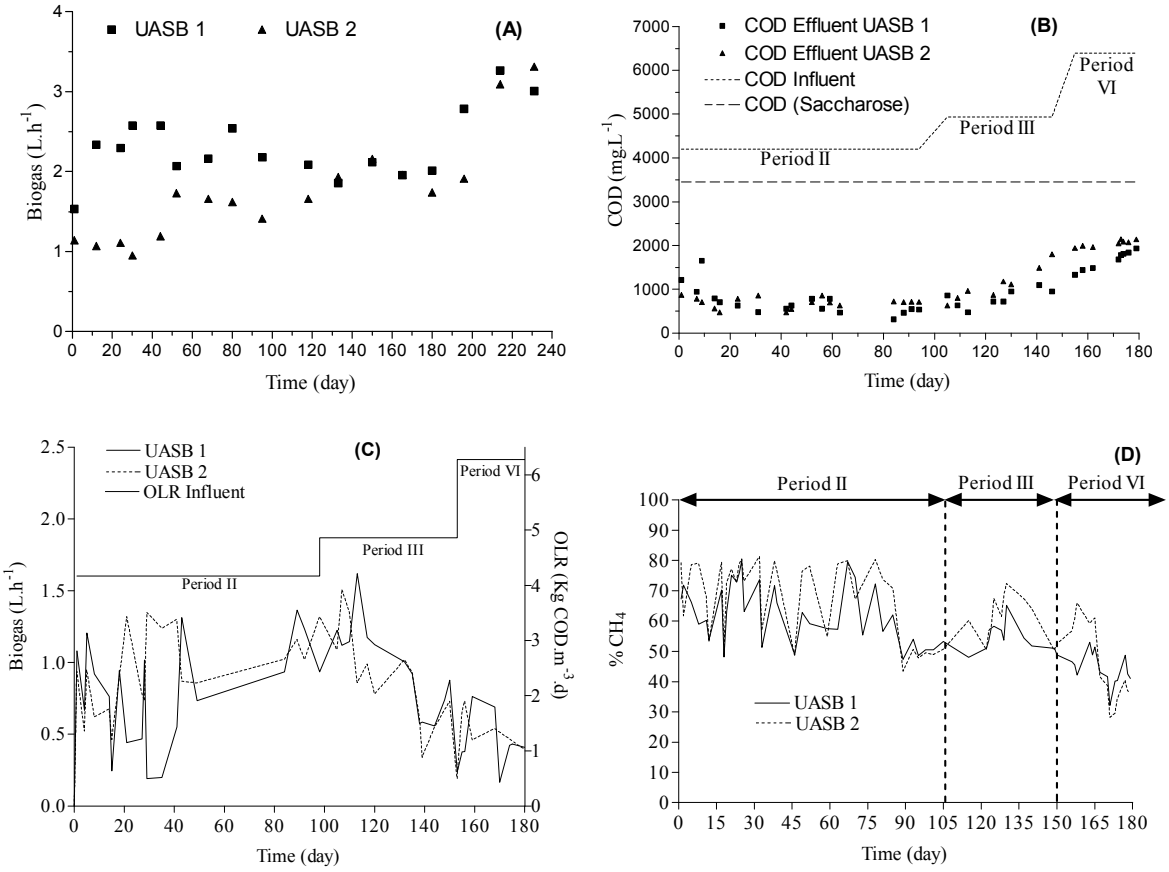

Figure 3: Biogas production vs time (A); COD effluent in UASB 1 and 2, during period I (fed only with saccharose); COD effluent of UASB 1 and 2 (fed with saccharose plus sodium oleate (UASB 1) and saccharose plus sodium stereate (UASB 2) (B); Biogas production in UASB 1 and 2 during periods II, III and IV (C); methane percentage in the biogas vs time in UASB 1 and 2, during periods II, III and IV (D).

adapted to the synthetic substrate at the end of 230 days of operation, since during this period there was an increase in the biogas production rate (Figure 3A) and in methane concentration in the biogas (Table 4). VS concentration in the anaerobic sludge decreased at the end of period I from 62.53 $g.L^{-1}$ (inoculum) to 30.58 $g.L^{-1}$ and 26.60 $g.L^{-1}$ in UASB 1 and 2, respectively. After starting the inoculation, the disaggregation of the sludge granules began and it was lost through the effluent because of the new operational conditions to which the anaerobic sludge was submitted.

UASB 1 and 2 – Periods II, III and IV – Substrate: Saccharose + LCFA

The COD of the influent applied during period I was increased in the subsequent periods by the added LCFA according Table 1, while the HDT was maintained at 24 h. Table 5 shows the results during steps II, III and IV. The lowest values of effluent alkalinity were observed in UASB 2, during period IV. The percent removal of COD, production of biogas ($L.h^{-1}$) and percent CH_4 in the biogas decreased considerably in all reactors with the increase in LCFA concentration (Figure 3. (A-D) and Table 4). SMA also declined at the end of each period, while the biomass in reactor UASB 2 had undetectable levels of SMA at the end of period IV (Table 5).

Table 4: Performance of the UASB reactors during period I using saccharose with substrate.

	UASB 1	UASB 2
COD Effluent ($mg.L^{-1}$)	400 - 1.319	300 - 1.264
Mean COD removal (%)	81.1	78.3
Biogas Production ($L.h^{-1}$)	1.53 - 3.26	0.95 - 3.31
Methane (%)	42.1 - 71.2	29.8 - 86.2

Table 5: Performance of the UASB reactors during periods II, III and IV.

	UASB 1			UASB 2		
Period[a]	**II**	**III**	**IV**	**II**	**III**	**IV**
pH Effluent	6.8-7.8	6.9-7.6	6.9-7.6	6.9-7.4	7.1-7.5	6.7-7.7
Alkalinity ($mg.L^{-1}$) $CaCO_3$)	1066-1804	1061-1148	1143-1374	1023-1364	1085-1229	715-1196
COD Effluent ($mg.L^{-1}$)	313-1652	475-1097	1332-1931	475-871	633-1800	1940-2143
COD Removal (%)	92.5-60.7	90.3-77.6	68.3-79.0	88.7-79.3	87.1-63.2	60.4-56.2
SMA (gCOD) $CH_4.g^{-1}$ $STV.d^{-1}$)	0.0040	0.0040	0.0021	0.0050	0.0030	ND[a]
Biogas ($L.h^{-1}$)	0.44-1.31	0.57-1.62	0.16 - 0.76	0.46-0.30	0.34-1.51	0.19-0.73
Methane (%)	48.1-79.9	40.0-65.2	32.1-53.0	53.0-81.3	50.3-72.4	28.2-66.0
Total VFA ($mg.L^{-1}$)	98.4-146.5	138.6-187.6	129.5-201.6	100.5-166,5	188.4-223.5	178.4-231.3

The COD removal when the reactors operated only with saccharose (Period I) averaged 80% (Table 4). Alkalinity and pH remained stable. The alkalinity observed was associated with alkalinity-generating substances from synthetic substrate used. In the following period (II) the OLR was increased to 4.16 kg COD. $m^{-3}.d^{-1}$ by addition of 250 $mg.L^{-1}$ sodium oleate (UASB 1) and 4.19 kg COD. $m^{-3}.d^{-1}$ by addition of 250 $mg.L^{-1}$ sodium stereate (UASB 2). Beginning in this period there was a decrease in effluent quality in the reactors, even though no solids loss was seen. After 24 h of operation, precipitates formed at the base of UASB 2.

In percentage terms, COD removal was similar to period I, although effluent quality decreased considerably in all the reactors (Figure 2B). Gas production was also found to be affected by added LCFA (Figure 2D) and the percent of CH_4 in the biogas decreased gradually in all the reactors. COD removal decreased progressively as the concentration of LCFA was increased to 500 $mg.L^{-1}$ (Period III) and 1000 $mg.L^{-1}$ (Period IV). This effect was more pronounced from period III because of the inhibition of the acetogenic bacteria involved in β-oxidation of fatty acids, affecting the overall performance.

The effect of COD removal was in part due to adsorption on the granules of the sludge. The pH was stable during the experiment, but the alkalinity decreased with the increased addition of LCFA, and the concentration of total VFA increased (Table 5). However, there was no acidification in the reactors, probably because of the neutralization of the VFA by the alkalinity of LCFA salts; hence the alkalinity observed was not associated with alkalinity-generating substances (mainly sodium acetate) in the substrate, but was generated from a source in the synthetic substrate. Our results suggest that the alkalinity observed is due to the presence of bicarbonate and alkalinity used to neutralize the VFA, since the operation of the reactors (6.8 to 7.2), does not allow hydroxide alkalinity to appear, which occurs at a pH higher than 8.3. A possible effect of alkalinity generated from the formation of ammonium bicarbonate must be considered, due to the ammonium chloride present in the synthetic effluent which, combined with the carbonic acid present in the medium, may form ammonium bicarbonate, helping maintain the pH in a stable range.

The SMA decreased with the increase of LCFA in all reactors, reaching undetectable levels in UASB 2, fed with sodium stereate. This suggests that sodium stereate is the main inhibitor, probably because of its greater hydrophobicity, which reduced the substrate-microorganism interaction. Rather than inhibition, flotation of granular biomass, leading to washout, was the most important operational problem observed in UASB 2. Lalman & Bagley (2001) found that oleic acid was anaerobically degraded at 21 ºC but stearic acid degradation was very slow in unacclimated cultures. Also in this case the biomass used for these experiments was enriched before LCFA addition using glucose, while in our case we used saccharose, in the absence of oleic or stearic acids. However, the authors used LCFA concentrations between 10-100mg/L, while in this study the concentrations were higher ((250-1000 mg/L).

The adsorption of LCFA to the sludge granules may have led to trapping biogas within the granules, modifying their density and leading to washout during periods III and IV. Physically, this effect can be observed through the heterogeneity in the granule sedimentation characteristics at the end of period III. Moreover, visually the occurrence of white granules was also observed, probably due to the adsorption of LCFA on the surface, similar to that observed by HWU *et al* (1998).

CONCLUSIONS

The results show that the use of the Griffith tube coupled to a particle analyser proved to be an effi-

cient method for the analysis of physical sedimentation characteristics of anaerobic sludge granules. It allowed the alterations in settling velocity resulting from substrate modification to be measured. Also the addition of LCFA to the substrate led to changes in the physical structure of the granules, reducing settling velocity at the same time that mean particle diameter was increased.

In our study the addition of LCFA altered the sedimentation profile of the granules with sedimentation velocities less than 0.83 $cm.s^{-1}$, as a consequence of the adsorption of LCFA. The addition of LCFA also caused a decrease in the overall performance of the reactors. This effect was more evident in the presence of sodium stereate, and led to total washout of the biomass at the end of the experiment.

The sedimentation velocity of sludge granules can be analysed using the Griffith Tube to determine the upper limit of influent input rates for a specific sludge in UASB reactors. The measurements can be used to show how the behaviour changes with time and operational conditions.

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