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A SIMPLIFIED ANALYSIS OF GRANULE BEHAVIOR IN ASBR AND UASB REACTORS TREATING LOW-STRENGTH SYNTHETIC WASTEWATER

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Abstract - This work presents an analysis of the changes observed in granule characteristics of sludge in the treatment of synthetic wastewater at a concentration of about 500 mgCOD/L in batch, fed-batch (ASBR) and continuous (UASB) bench-scale reactors under similar experimental conditions. Physical and microbiological properties of the granules were characterized as average particle size and sedimentation time and by optical and epifluorescence microscopy. Several samples were analyzed in order to identify the morphologies. Granules from sequencing batch and fed-batch reactors, either with or without mechanical mixing, did not undergo any physical or microbiological changes. However, during the experiment granules from the UASB reactor agglomerated due to the formation and accumulation of a viscous material, probably of microbial origin, when operated at low superficial velocities (0.072, 0.10 and 0.19 m/h). When the superficial velocity was increased to 8.0-10.0 m/h by means of liquid-phase recirculation, the granules from the UASB reactor underwent flocculation and the microbiological characteristics changed in such a way that the equilibrium of microbial diversity in the inoculum was not maintained. As a result, the only reactor that maintained efficiency and good solids retention during the assays was the ASBR, showing that there is a correlation between maintenance of microbial diversity and operating mode in the case of anaerobic treatment of low-strength wastewaters.
Keywords: Granule features; ASBR; UASB; Low-strength wastewater; Anaerobic treatment.

INTRODUCTION

Up-flow anaerobic sludge blanket (UASB) reactors are certainly the most commonly used anaerobic reactors worldwide, while anaerobic sequencing batch reactors (ASBR) have been pointed to as a potential system for wastewater treatment (Dague et al., 1992; Jeison and Chamy, 1999; Zaiat et al., 2001). The performance and stability of these reactors depend a lot on sludge granulation, but the success of the self-immobilization process is not warranted, since several factors affect this process (Kalyuzhnyi et al., 1996; Hulshoff-Pol et al., 1983). According to Liu et al. (1993), the UASB reactor is highly dependent on the granulation process, unlike anaerobic sequencing batch reactors.

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The granulation process, in which nondiscrete flocculent biomasses form discrete well-defined granules, is fundamental for the success of anaerobic systems that operate without inert support for biomass adhesion and biofilm formation (Kato et al., 1984, 1997). High settling velocities and high specific activity are the characteristics that are most important for efficient biomass retention in the reactor and high waste biodegradation (Lettinga et al., 1980; Hulshoff-Pol et al., 1983). Anaerobic granular sludge consists of dense multi-species microbial communities. The close association and interaction of anaerobic microorganisms in a granular system permit efficient transport and consumption of intermediate metabolites.

In ASBR reactors the flocculent biomass is gradually converted into highly active granular biomass that settles well due to a selection process during the decanting cycle. The decanting process causes washing out of the poorly settling flocs and disperse organisms, selecting the heavier, better-settling aggregates (Sung and Dague, 1995).

This article aims at evaluating anaerobic granule characteristics in two types of reactors treating a synthetic low-strength wastewater: an ASBR with mechanical mixing and an UASB reactor operated at low and high superficial velocities. The reactors were assayed under similar operating conditions and microscopic observations as well as some physical analyses were used to assess the granule characteristics in both reactors.

MATERIALS AND METHODS

The experimental apparatus (Figure 1) used in this study consisted of two bioreactors: (a) an ASBR with a work volume of 5 L treating 2 L wastewater per cycle (diameter of 18 cm and height of 26 cm, with a liquid height of 20 cm), equipped with a six-vertical-blade disk turbine impeller for mixing, operated in batch and fed-batch sequencing mode; and (b/c) an UASB reactor subject to low and high superficial velocities, respectively, operated in a continuous mode with a work volume of 1.3 L (diameter of 5.3 cm and height of 62 cm – reaction and sedimentation region), equipped without (b) and with (c) an external pump for mixing. The reactors were operated at 30 ± 2 °C. The ASBR was operated with sequential batch cycles of 8 and 6 hours, and the UASB reactor was operated with hydraulic detention times (HRT) of 8, 6 and 4 hours.

The operating conditions are shown in Tables 1, 2 and 3 for the ASBR, the UASB reactor operated at both low and high superficial velocities, respectively.

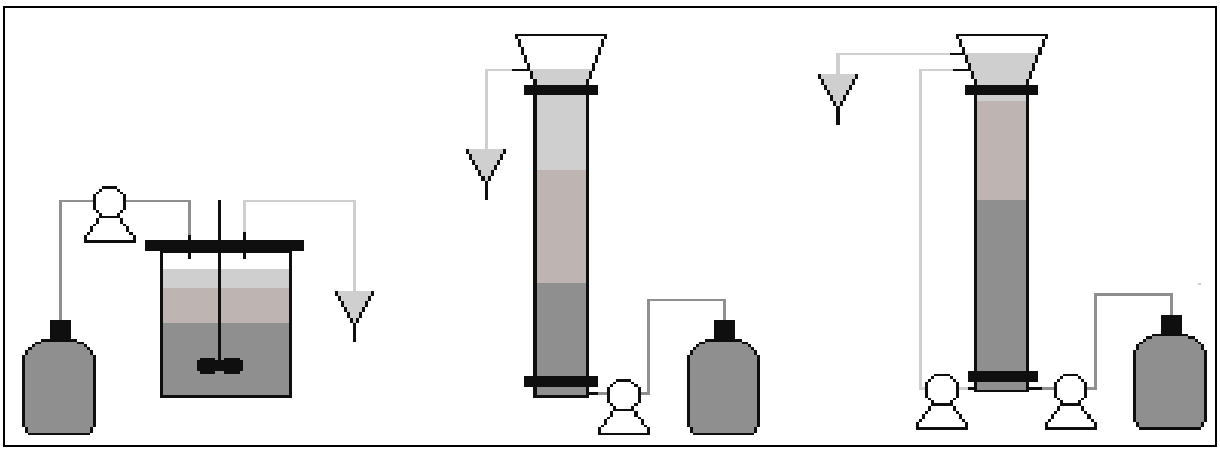
The two reactors were inoculated with a granular anaerobic sludge from a pilot UASB reactor treating synthetic sewage containing 52.3 mg-ts/g-sludge and 42.3 mg-tvs/g-sludge (81% mg-tvs/mg-ts), with an average particle size diameter of 2.3 ± 0.2 mm and a time required for settling out half of the sludge (θ_T) of approximately 10 s.

This concept of settling time has been previously proposed by Hulshoff-Pol et al. (1984) to analyze granular sludge settling characteristics. Initially, 100 mL of sludge was poured into a graduated cylinder. The cylinder was mixed in order to homogenize the sludge and was left to stand. The time required for settling out half of the sludge was measured visually using a chronometer. The assay was done in triplicate.

The synthetic wastewater with a chemical oxygen demand (COD) concentration of approximately 500 mg/L was prepared with sucrose (35 mg/L), starch (114 mg/L), cellulose (34 mg/L), meat extract (208 mg/L), soy oil (51 mg/L), NaCl (250 mg/L), $MgCl_2.6H_2O$ (7.0 mg/L), $CaCl_2.2H_2O$ (4.5 mg/L), $NaHCO_3$ (200 mg/L) and commercial detergent in order to emulsify the soy oil (3 drops/L). The medium was sterilized (121 °C, 15 min) in order to maintain its characteristics during the time of the experiment.

Concentrations of substrate (measured as COD) in unfiltered and filtered samples, total volatile acids (TVA), bicarbonate alkalinity (BA), total solids (TS), total volatile solids (TVS), total suspended solids (TSS), volatile suspended solids (VSS) and pH were monitored in both the influent and effluent for all conditions, according to the Standard Methods for the Examination of Water and Wastewater (1995).

The microbiological tests of the anaerobic sludge were done using an *Olympus® BX 60-FLA* microscope equipped with an *Optronics* digital camera. The bioparticles were washed with distilled water and drops of the washwater were immediately examined by phase contrast microscopy. Also samples were examined on glasses slides covered with 2% agar film. Fluorescence was observed by using an ultraviolet light source. Images were acquired using *Image Pro-Plus® 3.0.1* software.



(a) (b) (c)

Figure 1: Scheme of the reactors: (a) ASBR, (b) UASB and (c) UASB with circulation.

RESULTS AND DISCUSSION

Tables 1 to 3 show a summary of the results obtained in the assays with the ASBR and with the UASB reactor operated at low and high superficial velocities, respectively.

The sludge used in the two reactors in all assays had good sedimentation and microbiological properties, as can be seen by the average granule size ($\phi_G = 2.3 \pm 0.2$ mm), by the time required for settling out half of the sludge ($\theta_T \cong 10$ s) and by the microbiological population distribution (Figure 2 and Table 4), which showed great morphological variability of bacteria and methanogenic archaea cells, suitable for the experiments developed.

Table 4 and Figure 3 show the microbiological characteristics of the sludge after batch and fed-batch operation, in which the effects of mixing and feed strategy, respectively, were studied (also see Table 1). Analysis of these tables and figures shows that both the granule characteristics ($\phi_G = 2.6 \pm 0.4$ mm and $\theta_T \cong 10$ s) and microbiological diversity were maintained, despite the different mixing rates (0, 25, 50 and 75 rpm) and feed modes (Rodrigues et al., 2003-a, 2003-b). The granules obtained from the ASBR had morphologies similar to those encountered in the sludge used as inoculum, indicating that morphology was maintained, despite the fact that this sludge was obtained from a continuous reactor treating a wastewater different from that used in the current research.

The results obtained in the assays with the UASB reactor, operated at low up-flow velocities (0.072, 0.10 and 0.15 m/h), are shown in Table 2. It should be pointed out that bed movement was considerably reduced, probably due to the low up-flow rate in relation to the apparent sludge density.

Thus, it can be seen that together with a system efficiency which as lower ASBR, than that of the granule characteristics of the sludge, i.e., the average diameter and settling time, were maintained ($\phi_G = 2.7 \pm 0.5$ mm and $\theta_T \cong 30$ s). However, generation and accumulation of a yellowish viscous material, probaby of biological origin, were observed on the surface of the granules. This material caused agglomeration of the granules, thus resulting in a gradual accumulation of biogas and the consequent flotation of the sludge bed with low solids retention, which caused some loss of sludge. This behavior occurred for the three hydraulic detention times applied. It should be pointed out that accumulation of this viscous material started at the top of the sludge blanket, propagating extending the reactor inlet, i.e., towards the bottom of the blanket, probably due to substrate availability (starvation phenomenon).

Neither generation nor accumulation of this viscous material was observed in the ASBR without mechanical mixing and an eight-hour cycle. When the UASB reactor was operated at low up-flow velocities the sludge was not submitted to microbiological analysis at the end of the experiment.

Results for operation of the UASB reactor at high up-flow velocities obtained in assays performed to verify the effect of hydraulic detention time are

shown in Table 3, where a performance similar to that of the UASB reactor at low velocities, but not as good as that of the ASBR, can be seen. The microbiological characteristics are shown in Table 4 and in Figures 4 and 5. It should be mentioned that external liquid-phase recirculation due bed movement, visually much better than that observed in the UASB reactor without recirculation, but not as good as that in the ASBR with a mixing rate of 50 rpm (determined with the best mixong value by Rodrigues et al., 2003-a).

During operation of this reactor significant changes in the sludge granule characteristics were observed. The top of the bed was found to be typically flocculent (less than 0.2 mm and θ_T greater than 1 min), whereas the bottom of the bed was found to be typically granular (ϕ_G = 2.6 ± 0.4 mm and $\theta_T \cong$ 30 s). As a result, it was necessary to gradually reduce the superficial velocity to maintain solids retention.

Table 1: Mean values of variables monitored in the ASBR reactor

	C_{ET} [(a)] (mgCOD/L)	C_{ES} [(a)] (mgCOD/L)	BA [(b)] ($mgCaCO_3$/L)	TVA [(b)] (mgHAc/L)	pH [(b)]	VSS [(c)] (mg/L)
Influent	486 ± 26	–	128 ± 4	35 ± 4	8.8 ± 0.3	22 ± 10
Effluent (6 h-batch assays)	94 ± 9	62 ± 3	222 ± 5	22 ± 2	6.9 ± 0.1	28 ± 14
Effluent (8 h-batch assays)	102 ± 16	71 ± 7	232 ± 7	25 ± 6	6.9 ± 0.1	46 ± 24
Effluent (6-fed-batch assays)	109 ± 14	76 ± 9	230 ± 17	24 ± 3	7.0 ± 0.1	26 ± 11

Number of samples (minimum): [(a)] 16, [(b)] 9, [(c)] 7;
Batch assays: θ_B = 6 h; VOL = 778 mgCOD/L.d; SOR = 28.3 mgCOD/g-tvs.d; θ_A = 21 d; mixing rate = 50 rpm; X = 110.7 g-tvs;
Batch assays: θ_B = 8 h; VOL = 583 mgCOD/L.d; SOR = 20.8 mgCOD/g-tvs.d; θ_A = 31 d; mixing rate = 0-75 rpm; X = 110.7 g-tvs;
Fed-batch assays: θ_B = 6 h; VOL = 778 mgCOD/L.d; SOR = 35.9 mgCOD/g-tvs.d; θ_A = 28 d; mixing rate = 50 rpm; X = 84.0 g-tvs.

Table 2: Mean values of variables monitored in the UASB reactor at low superficial velocities.

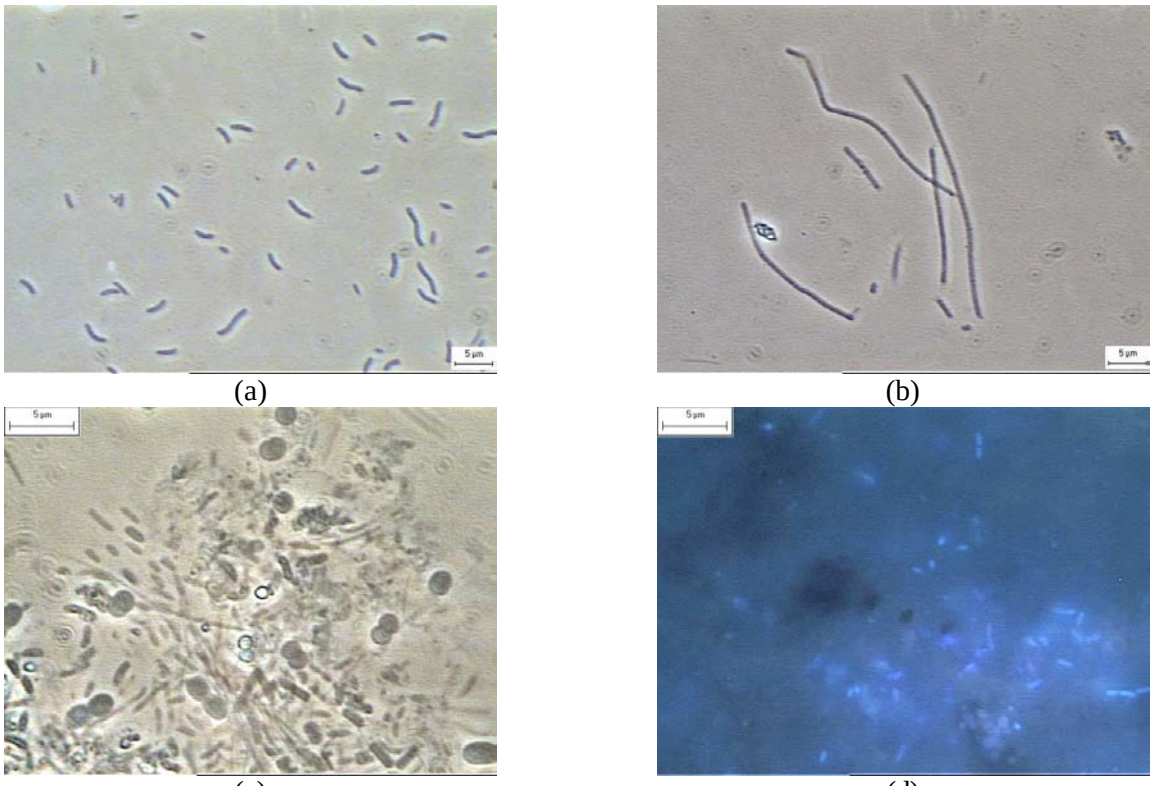
	C_{ET} [(a)] (mgCOD/L)	C_{ES} [(a)] (mgCOD/L)	BA [(b)] ($mgCaCO_3$/L)	TVA [(b)] (mgHAc/L)	pH [(b)]	VSS [(c)] (mg/L)
Influent	485 ± 27	–	126 ± 7	34 ± 4	9.0 ± 0.1	31 ± 16
Effluent (θ_H = 8 h)	127 ± 21	93 ± 9	215 ± 20	24 ± 4	7.5 ± 0.1	18 ± 8
Effluent (θ_H = 6 h)	144 ± 18	121 ± 16	195 ± 6	31 ± 5	7.3 ± 0.1	20 ± 13
Effluent (θ_H = 4 h)	183 ± 24	158 ± 23	184 ± 16	42 ± 8	7.2 ± 0.1	26 ± 13

Number of samples (minimum): [(a)] 32; [(b)] 10; [(c)] 5;
θ_H = 8 h: VOL = 1.49 gCOD/L.d; SOR = 120 mgCOD/g-tvs.d; θ_A = 35 d; v_S = 0.072 m/h; X = 12.0 g-tvs;
θ_H = 6 h: VOL = 1.96 gCOD/L.d; SOR = 145 mgCOD/g-tvs.d; θ_A = 31 d; v_S = 0.10 m/h; X = 12.3 g-tvs;
θ_H = 4 h: VOL = 2.86 gCOD/L.d; SOR = 199 mgCOD/g-tvs.d; θ_A = 32 d; v_S = 0.15 m/h; X = 11.4 g-tvs.

Table 3: Mean values of variables monitored in the UASB reactor at high superficial velocities.

	C_{ET} [(a)] (mgCOD/L)	C_{ES} [(a)] (mgCOD/L)	BA [(b)] ($mgCaCO_3$/L)	TVA [(b)] (mgHAc/L)	pH [(b)]	VSS [(c)] (mg/L)
Influent	444 ± 51	–	134 ± 5	26 ± 2	8.9 ± 0.4	75 ± 36
Effluent (θ_H = 8 h)	119 ± 15	98 ± 16	201 ± 45	16 ± 2	7.0 ± 0.1	19 ± 6
Effluent (θ_H = 6 h)	143 ± 25	106 ± 10	207 ± 9	16 ± 1	6.9 ± 0.2	35 ± 9
Effluent (θ_H = 4 h)	181 ± 40	130 ± 25	220 ± 20	23 ± 1	7.7 ± 0.1	32 ± 9

Number of samples (minimum): [(a)] 18, [(b)] 16, [(c)] 7;
θ_H = 8 h: VOL = 1.33 gCOD/L.d; SOR = 133 mgCOD/g-tvs.d; θ_A = 28 d; v_S = 10.7 m/h; X = 9.5 g-tvs;
θ_H = 6 h: VOL = 1.78 gCOD/L.d; SOR = 177 mgCOD/g-tvs.d; θ_A = 30 d; v_S = 8.2-1.3 m/h; X = 8.9 g-tvs;
θ_H = 4 h: VOL = 2.66 gCOD/L.d; SOR = 255 mgCOD/g-tvs.d; θ_A = 20 d; v_S = 9.8 m/h; X = 8.0 g-tvs.



(a) (b)

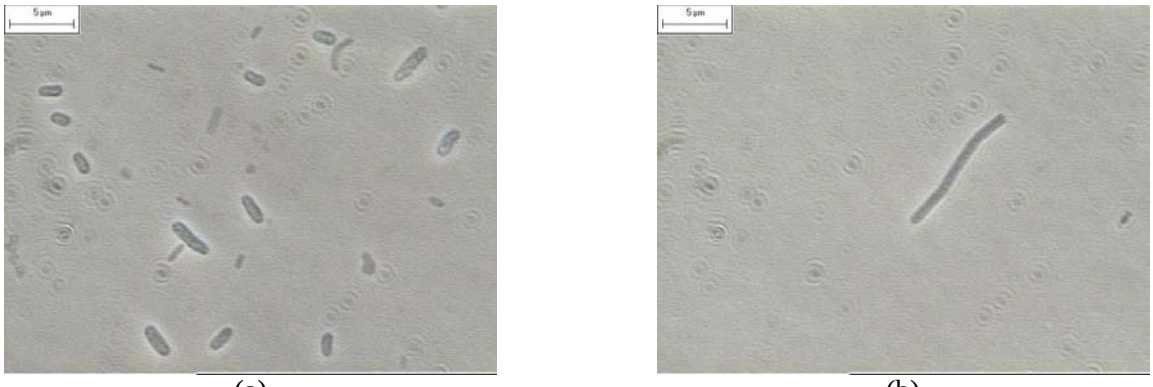
(c) (d)

Figure 2: Photomicrography of the sludge used as inoculum in all reactors (a – rods; b – *Methanosaeta*-like; c – cocci; d – cocci and rod-like archaeal cells).

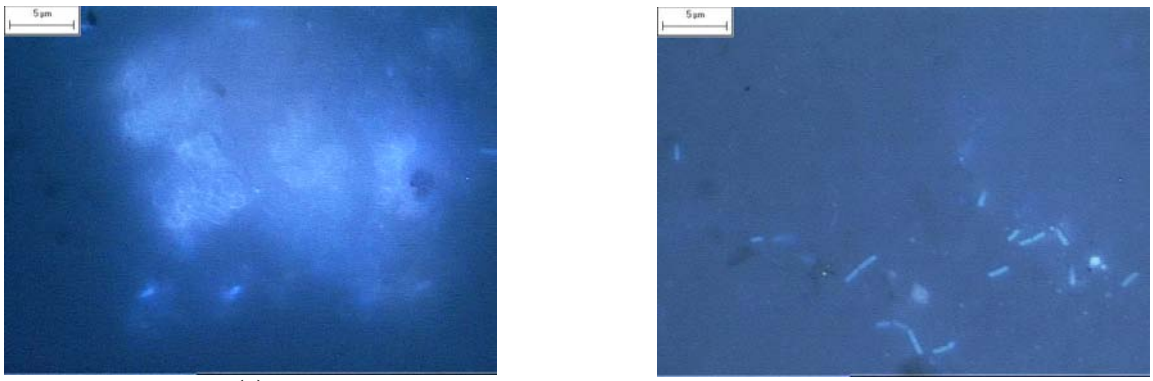
Table 4: Microbiological qualitative analysis of the sludge at the end of the experiment.

	Inoculum	**ASBR**	**UASB* [1]**	**UASB* [2]**
Rods	++++	++++	++++	++++
Cocci	++	+++	++++	++++
Fluorescent cocci	++	++	+++	+++
Fluorescent rods	++	++	+++	+++
Vibrium	+	++	++	++
Methanosarcina-like archaeal cells	+	++	–	–
Methanosaeta-like archaeal cells	+++	++	+	++
Filaments	++	++	++	++++

Notation: *Reactor with liquid recirculation; [1] – top of the blanket; [2] – bottom of the blanket;
++++ predominant; +++ present in large numbers; ++ present; + rare; – absent.

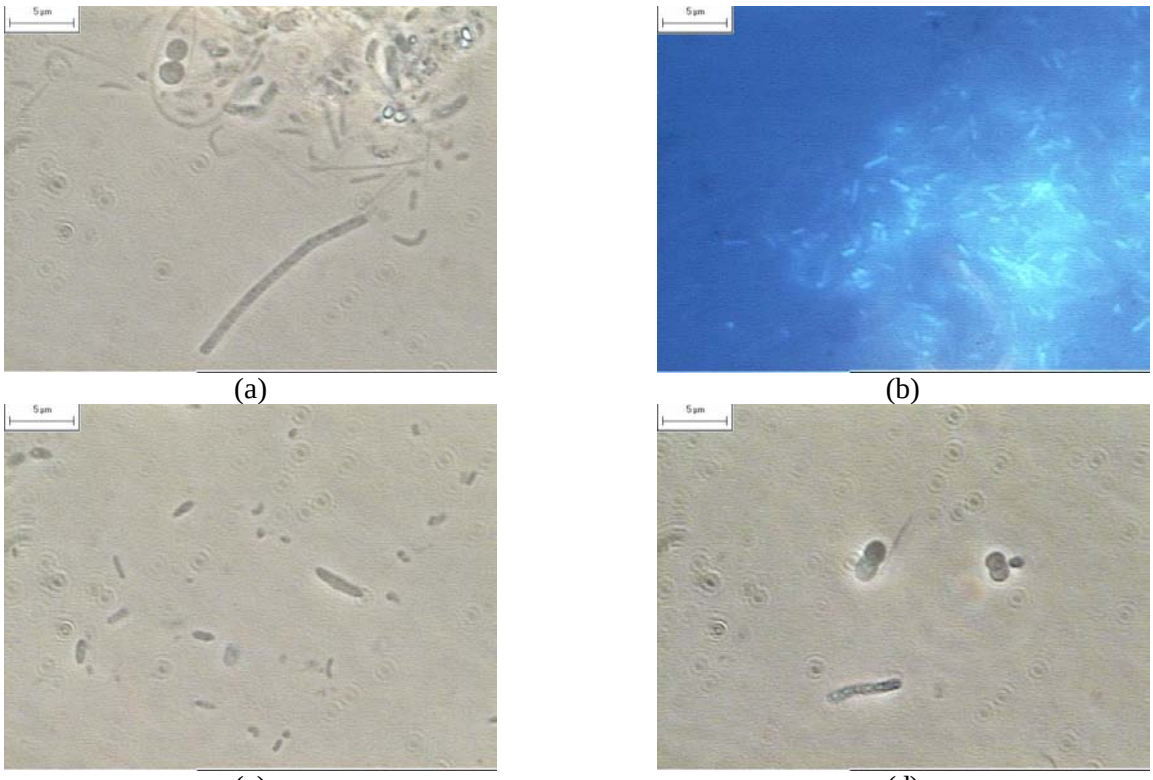


(a) (b)



(c) (d)

Figure 3: Photomicrography of the sludge used in ASBR reactor (a – rods; b – filaments; c – *Methanosarcina*-like; d – cocci and rod-like archaeal cells).

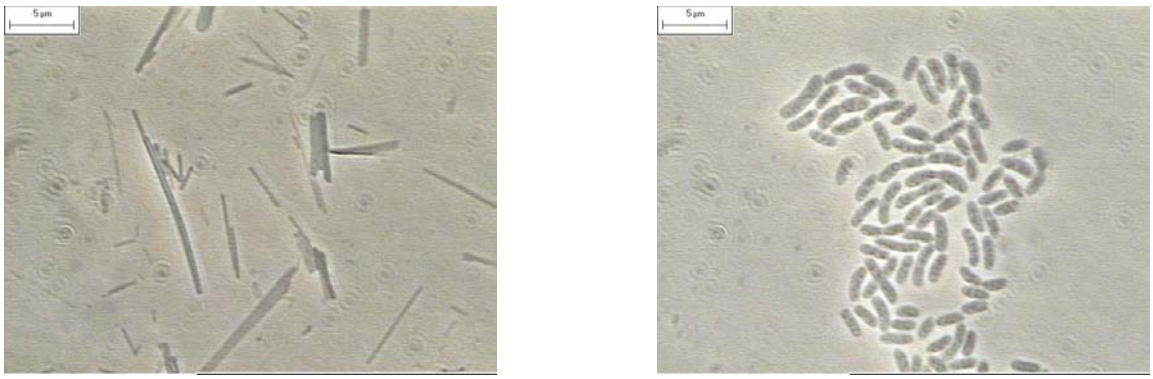


(a) (b)

(c) (d)

Figure 4: Photomicrography of the sludge at the top of the blanket used in UASB reactor at high superficial velocities (a – filaments; b – rod-like archaeal cells; c – rods; d – cocci).

(a) (b)



(c) (d)

Figure 5: Photomicrography of the sludge at the bottom of the blanket used in UASB reactor at high superficial velocities (a, b – *Methanosaeta*-like; c – filaments; d – rods).

Microbiological analyses performed at the end of each experiment indicate that stratification of the bed reactor based on morphological composition took place. At the bottom of the reactor, bacilli, cocci and filaments as well as fluorescent cocci and bacilli and *Methanosaeta*-like archaeal cells predominated, indicating the presence of methanogenic archaeal cells. At the top of the reactor the morphologies were similar to those observed at the bottom, but with fewer *Methanosaeta*-like cells and filaments. This may be directly related to the differences observed in the granules. Fang et al. (1994) verified that acetoclastic Methanosaeta were the key structural granules from UASB reactor treating different substrates. Filamentous microorganisms and *Methanoseata*-like cells seem to have a structural role, producing stability of the pellets formed.

It should be pointed out that changes in the granule characteristics also started at the top of the sludge blanket and extended towards the bottom of the reactor, probably related to substrate availability (starvation phenomenon). In comparative terms, change in granule shape did not take place in the ASBR at a mechanical mixing rate of 75 rpm and an eight-hour cycle, i.e., under similar operating conditions and severe hydrodynamic conditions.

CONCLUSIONS

The results obtained in this work allowed identification of the possible effect of reactor operation mode, continuous or batch, in treating low-strength synthetic wastewater on preservation of granule size and microbiological characteristics. A more stable behavior was observed when substrate became less available, which is characteristic of batch operation (ASBR) and did not occur in the continuous mode, despite the hydrodynamic conditions of the system.

The granulated sludge underwent little morphological change in either morphological or physical features (sedimentation time and granule size) during ASBR operation. On the other hand, the sludge was change considerably modified during UASB reactor operation, forming two quite distinct zones with different morphological and physical characteristics.

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NOMENCLATURE

Symbols

BA	bicarbonate alkalinity,	$mgCaCO_3/L$
C_{ES}	concentration of filtered substrate in the effluent,	mgCOD/L
C_{ET}	concentration of unfiltered substrate in the effluent,	mgCOD/L
C_I	concentration of unfiltered substrate in the influent,	mgCOD/L
SOR	specific organic removal	mgCOD/

	using concentration of unfiltered effluent,	g-tvs.d
TVA	concentration of total volatile acid,	mgHAc/L
TVS	concentration of total volatile solids,	mg/L or mg/g-sludge
VOL	volumetric organic loading,	mgCOD/L.d
v_S	superficial velocity,	m/h
VSS	concentration of volatile suspended solids,	mg/L or mg/g-sludge
V_T	work volume of the reactor (discontinuous and continuous),	L
V_{TPC}	volume treated per cycle (discontinuous reator),	L/d
X	amount of biomass,	g-tvs

Greek Symbols

ϕ_G	diameter of the granules,	mm
θ_A	total period of the assay,	d
θ_B	batch time,	h
θ_{FB}	fed-batch time,	h
θ_H	hydraulic residence time,	h
θ_T	time required for settling out half of the sludge,	min

Abbreviations

ASBR	anaerobic sequencing batch reactor	(-)
COD	chemical oxygen demand	(-)
tvs	total volatile solids	(-)
ts	total solids	(-)
UASB	up-flow anaerobic sludge blanket	(-)

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