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Effects of Feed Time, Organic Loading and Shock Loads in Anaerobic Whey Treatment by an AnSBBR with Circulation

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Abstract The aim of this work was to investigate the effect of different feeding times (2, 4, and 6 h) and organic loading rates (3, 6 and 12 gCOD l^{-1} day^{-1}) on the performance of an anaerobic sequencing batch reactor containing immobilized biomass, as well as to verify the minimum amount of alkalinity that can be added to the influent. The reactor, in which mixing was achieved by recirculation of the liquid phase, was maintained at 30±1°C, possessed 2.5 l reactional volume and treated 1.5 l cheese whey in 8-h cycles. Results showed that the effect of feeding time on reactor performance was more pronounced at higher values of organic loading rates (OLR). During operation at an OLR of 3 gCOD l^{-1} day^{-1}, change in feeding time did not affect efficiency of organic matter removal from the reactor. At an OLR of 6 gCOD l^{-1} day^{-1}, reactor efficiency improved in relation to the lower loading rate and tended to drop at longer feeding times. At an OLR of 12 gCOD l^{-1} day^{-1} the reactor showed to depend more on feeding time; higher feeding times resulted in a decrease in reactor efficiency. Under all conditions shock loads of 24 gCOD l^{-1} day^{-1} caused an increase in acids concentration in the effluent. However, despite this increase, the reactor regained stability readily and alkalinity supplied to the influent showed to be sufficient to maintain pH close to neutral during operation. Regardless of applied OLR, operation with feeding time of 2 h was which provided improved stability and rendered the process less susceptible to shock loads.

Keywords AnSBBR · Organic loading rate · Shock load · Feeding time · Cheese whey

Notation

OLR	organic loading rate, gCOD l^{-1} day^{-1}
C_{TS}	organic matter concentration in unfiltered samples in the effluent, mgCOD l^{-1}

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C_{FS}	organic matter concentration in filtered samples in the effluent, mgCOD l^{-1}
C_{SAFL}	organic matter concentration in unfiltered samples in the influent, mgCOD l^{-1}
ε_{FS}	efficiency of organic matter removal for filtered samples in the effluent, %
ε_{TS}	efficiency of organic matter removal for unfiltered samples in the effluent, %
BA	bicarbonate alkalinity, mgCaCO$_3$ l^{-1}
TVA	concentration of total volatile acids, mgHAc l^{-1}
IVA	concentration of intermediate volatile acids (mg l^{-1})
TS	total solids concentration, mg l^{-1}
TVS	total volatile solids concentration, mg l^{-1}
STS	total suspended solids concentration, mg l^{-1}
SSV	suspended volatile solids concentration, mg l^{-1}
C_{CH4}	methane concentration in the biogas, mmol l^{-1}
C_{TVS}	total volatile solids concentration relative to the immobilized biomass, mgTVS g foam^{-1} or gTVS l reaction medium^{-1}
C_{TS}	total solids concentration relative to the immobilized biomass, mgTS g foam^{-1} or gTS l reaction medium^{-1}
C_{TVS}/ C_{TS}	ratio between total volatile solids and total solids concentrations relative to the immobilized biomass, gTVS gTS^{-1}
ROL	removed organic load, gCOD l^{-1} day^{-1}
ROL$_{FS}$	removed organic load for filtered samples in the effluent, gCOD l^{-1} day^{-1}
ROL$_{TS}$	removed organic load for unfiltered samples in the effluent, gCOD l^{-1} day^{-1}
V_A	volume fed or volume renewed per cycle, l;n|number of daily cycles, cycles day^{-1}
V_u	volume of reaction medium in the system, l
AnSBBR	anaerobic sequencing batch biofilm reactor
UASB	upflow anaerobic sludge blanket reactor
COD	chemical oxygen demand

Introduction

Anaerobic treatment of cheese whey can be used to minimize pollution problems caused by the disposal of untreated whey into waterways. However, although it is possible to treat this kind of effluent in high-rate anaerobic reactors, some characteristics of the whey, such as high biodegradability, lack of alkalinity and high organic load make this rather difficult [1]. Moreover, due to its high biodegradability, whey tends to acidify readily, which impairs maintenance of process stability when the reactor is operated at high organic loading rates (OLR). Hence, to maintain process stability, in most cases addition of some external source of alkalinity is required, such as bicarbonate, carbonate, or hydroxide [2]. More reliable operation strategies are also required that enable treatment with improved control on process stability.

With regard to continuous reactors, operation strategies such as recirculation of the effluent, for instance, are helpful in diluting the influent and, at the same time, increase available alkalinity, reducing or eliminating the need for an external alkalinity source [3, 4]. Some works in the literature also show that utilization of two-phase systems (acidogenic reactor followed by methanogenic reactor) enable stable operation without the need for alkalinity addition [5, 6]. With regard to the anaerobic sequencing batch biofilm reactor (AnSBBRs), some operation strategies such as longer feeding time, are important tools for gaining improved control over the process. Longer feeding time guarantees that the concentration of volatile acids, intermediates of anaerobic metabolism, does not reach high values, which may be harmful to the biomass [7–9].

However, there is a lack of investigations in the literature that deal with the behavior of this type of system when submitted to different feeding times and increasing OLR in the treatment of cheese whey. Optimization of the amount of alkalinity added is also of fundamental importance for minimizing operation costs.

Therefore, as a contribution to find solutions for the technological problems that this type of system still presents in the treatment of cheese whey and to optimize the operation of this system, this research was undertaken to assess the effect of feeding time, shock load, and alkalinity supplementation on the efficiency and stability of an anaerobic sequencing biofilm batch reactor (AnSBBR) with liquid-phase recirculation operated at different OLRs.

Materials and Methods

The reactor shown in Fig. 1, with a total capacity of 3.8 l consisted of an acrylic cylindrical column with a height of 540 mm, an external diameter of 100 mm and a wall thickness of 3.5 mm and was provided with an automatic unit for feeding, discharge, and recirculation of the liquid phase at a velocity of 0.19 cm s^{-1} [10]. The recirculation unit comprised (1) a lateral reservoir with total capacity of 2.1 l, consisting of a cylindrical acrylic container with a height of 300 mm, an external diameter of 100 mm and a wall thickness of 3.5 mm; and (2) a peristaltic pump with a maximum capacity of 30 l h^{-1}. A 100-ml measuring cylinder was attached to the recirculation system for flow rate measurements. Feeding and discharge were performed using diaphragm pumps equipped with automatic timers. The chamber in which the reactor remained was kept at 30 ± 1°C by a heating system composed of resistances and fans, as well as a temperature sensor and controller.

As immobilizing support, 1-cm polyurethane foam cubes were used. The support was placed between perforated stainless steel plates, which divided a height of 540 mm of the reactor in five parts to avoid bed compacting. A 20-mm compartment at the bottom of the reactor allowed enhanced distribution of the wastewater, preventing in this way formation of preferential routes. At the upper part of the reactor, a 40-mm region functioned as a biogas collector (CH_4 and CO_2). The inoculum used in all experiments came from an upflow anaerobic sludge blanket reactor treating wastewater from a poultry slaughterhouse. Total volume of reaction medium in the reactor-reservoir system amounted to 2.5 l; 0.9 l in the reactor and 1.6 l in the lateral reservoir. Synthetic wastewater was prepared by dissolving dehydrated cheese whey in tap water; experimentally, 1 g of dehydrated whey corresponded to about 1 g COD. In addition to cheese whey, the influent was supplemented with sodium bicarbonate and other salts: $NiSO_4 \cdot 6H_2O$ (0.830 mg l^{-1}), $FeSO_4 \cdot 7H_2O$ (4.20 mg l^{-1}), $FeCl_3 \cdot 6H_2O$ (0.420 mg l^{-1}), $CaCl_2 \cdot 2H_2O$ (39.2 mg l^{-1}), $CoCl_2 \cdot 6H_2O$ (0.067 mg l^{-1}), SeO_2 (0.058 mg l^{-1}), KH_2PO_4 (70.8 mg l^{-1}), K_2HPO_4 (18.0 mg l^{-1}) and Na_2HPO_4 (27.8 mg l^{-1}), based on influent cheese whey concentration of 1.67 gCOD l^{-1}. It should be mentioned that concentration of these salts was varied proportionally to the influent whey concentration which varied from 1.67 to 13.3 gCOD l^{-1}.

The monitored variables during reactor operation were: unfiltered (C_{TS}) and filtered (C_{FS}) organic matter concentration (as chemical oxygen demand—COD), bicarbonate alkalinity (BA), total volatile acids (TVA), total solids (TS), total volatile solids (TVS), total suspended solids(TSS) and volatile suspended solids (VSS), in addition to pH and volume

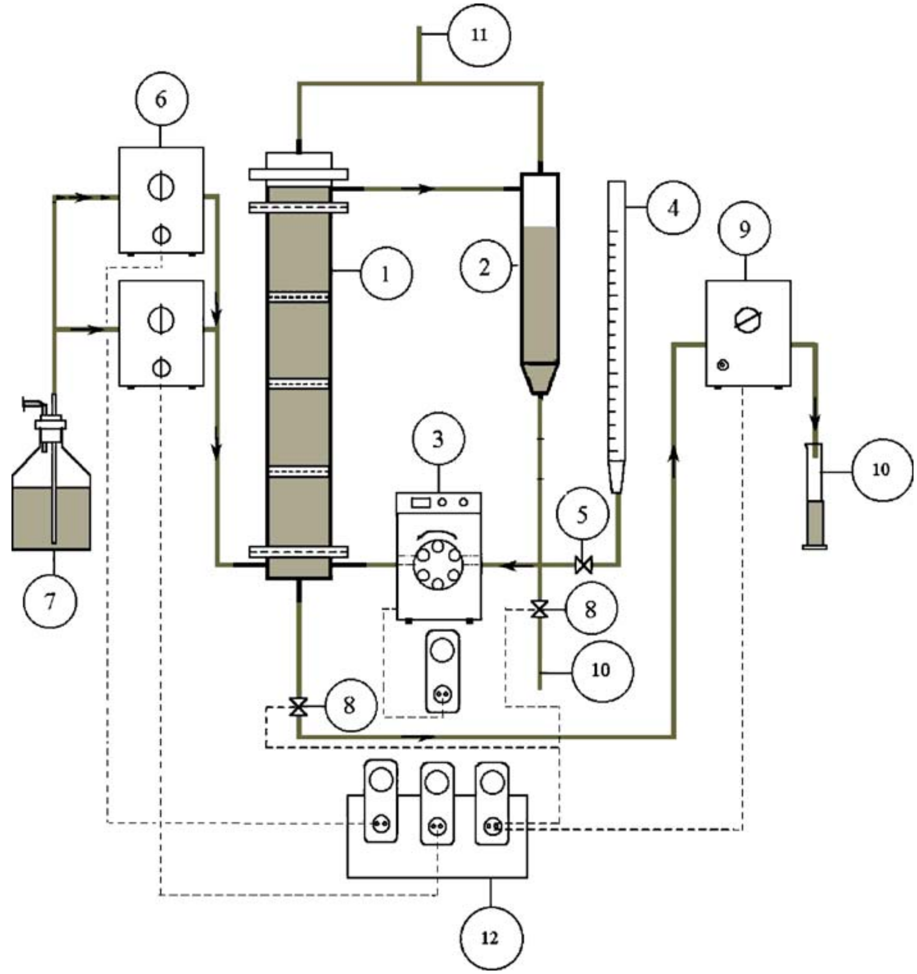


Fig. 1 Schematic representation of the system used for treating cheese whey [*1* reactor containing immobilized biomass; *2* circulation reservoir; *3* circulation pump; *4* flow rate meter; *5* valve; *6* feed pump; *7* synthetic wastewater reservoir; *8* discharge valves; *9* discharge pump; *10* effluent outlet; *11* biogas outlet; *12* control unit; *solid lines* hydraulic lines; *broken lines* power lines]

of discharged medium. Analyses were performed according to *APHA* [11]. The intermediate volatile acids (VA_I) were analyzed by gas chromatography using a Hewlett Packard® 6890 gas chromatography equipped with a flame ionization detector. The volume injected was 1 μl. Methane concentration in the biogas (C_{CH4}) was also analyzed by the same chromatograph equipped with a thermal conductivity detector.

Prior to the investigation of the effect of organic loading rate (OLR) on reactor stability and efficiency, some preliminary tests were performed (*Tests I, II, III* and *IV*). The objective of these tests was to verify if, for a same OLR, maintaining a residual volume inside the reactor at the end of each cycle, would improve process efficiency and stability in the treatment of cheese whey. In these tests, the reactor operated at an OLR of 12 gCOD l^{-1} day^{-1}, with supplementation of sodium bicarbonate to the influent at a proportion of 0.5 $gNaHCO_3$ per $gCOD_{fed}$ and 8-h cycles, totaling three cycles a day. To maintain a constant OLR of 12 gCOD l^{-1} day^{-1} in all tests, in each test, the cheese whey concentration in the influent was increased proportionally to the decrease in the ratio between volume fed in each cycle (V_A—which is equal to the discharged volume, i.e., volume treated per cycle) and total volume of reaction medium in the system (V_u—which remained constant at 2.5 l in all tests).

The test considered as reference (Test I) was carried out by feeding the reactor with 2.5 l of cheese whey at a concentration of 4 gCOD l^{-1}, i.e., in this test no residual volume remained. The remaining tests studied were: (II) feed volume of 1.75 l with residual volume of 0.75 l and influent organic matter concentration of 5.70 gCOD l^{-1}; (III) feed volume of 1.25 l with residual volume of 1.25 l and influent organic matter concentration of 8 gCOD l^{-1}; and (IV) feed volume of 0.63 l with residual volume of 1.87 l and influent organic matter concentration of 16 gCOD l^{-1}. As will be shown, Tests II and III were those that presented the best results. It was, hence, decided that the investigation of the effect of feeding time and OLR on reactor stability and efficiency would be performed at operation conditions intermediate to those used in these tests, i.e., feed volume per cycle of about 1.5 l and residual volume of 1.0 l.

The investigation of the effect of OLR and feeding time on reactor stability and efficiency was carried out by increasing the cheese whey concentration in the influent and by increasing the feeding time of the reactor. Three feeding times were investigated (2, 4, and 6 h) for three cheese whey concentrations in the influent (1.67, 3.34, and 6.67 gCOD l^{-1}, which corresponded to OLRs of 3, 6 and 12 gCOD l^{-1} day^{-1}). In addition, organic shock loads of 24.0 gCOD l^{-1} day^{-1} were applied at the end of each operation condition.

The investigation was carried out as follows: for the first OLR of 3 gCOD l^{-1} day^{-1}, at the beginning of each cycle recirculation of the residual medium (1.0 l) was initiated at a velocity of 0.19 cm s^{-1} for 470 min. Feeding of 1.5 l cheese whey, initiated simultaneously with the recirculation, occurred during a varied period. Feed strategy was established using constant flow rate. At the end of the cycle, recirculation was interrupted and 1.5 l effluent was subsequently discharged for 10 min. The remaining medium, denominated residual volume, was kept in the reactor. Feed strategy was established by the loading time of the reactor under the following conditions: (a) feed time of 120 minutes, characterizing fed-batch operation during 25% of the cycle; (b) feed time of 240 minutes, characterizing batch operation during 50% of the cycle; and (c) feed time of 360 minutes, characterizing fed-batch operation during 75% of the cycle.

Optimization of alkalinity supplementation in the influent was carried out as follows: for each regime, operation of the reactor was initiated with supplementation of $NaHCO_3$ in the influent at a 1:1 ratio of $gNaHCO_3$:$gCOD_{fed}$ and as soon as the system started showing stability of the main monitored variables, the $gNaHCO_3$:$gCOD_{fed}$ ratio in the influent was reduced so as to determine the minimum amount of alkalinity necessary for operational stability.

As soon as stability was reached profiles were run of the main monitored variables (C_{FS}, BA, TVA, IVA, C_{CH4}). The reactor was then submitted to one-cycle shock loads of 24 gCOD l^{-1} day^{-1}. Profiles of C_{FS}, BA, TVA and IVA were also run in the cycle subsequent to the shock load. In the cycles subsequent to the shock load the system was monitored with attention not only to verify whether efficiency and stability were maintained after the perturbation but also to verify the recovery time of the system after an eventual destabilization.

After the feed strategy test for the OLR of 3 gCOD l^{-1} day^{-1}, the same tests were run for 6 gCOD l^{-1} day^{-1} and 12 gCOD l^{-1} day^{-1}. At the end of each operational condition bioparticle samples were taken from the reactor for (1) microbiological analysis which was carried out by means of common optical and fluorescence phase contrast microscopy, using a BX41 Olympus® microscope, and for (2) analysis of the concentrations of total solids (S_{TS}) and total volatile solids (S_{TVS}) in the reactor. The latter analysis enables estimating the biomass concentration in the reactor in the form of total volatile solids (S_{TVS}).

The removed organic load (ROL) was calculated for each operational condition. ROL was defined as the amount of organic matter removed per unit of time and of wastewater volume in the reactor. For instance, gCOD l^{-1} day^{-1} for reactors operated in the batch mode the removed organic load for filtered (ROL_{FS}) and unfiltered samples (ROL_{TS}) of the effluent can be calculated through Eqs. (1) and (2), where C_{SAFL} is the concentration of organic matter in the influent, V_A is the volume of wastewater fed in the cycle, V_u is the volume of wastewater in the system (2.5 l), C_{FS} is the concentration of filtered organic matter in the effluent, C_{TS} is the concentration of unfiltered organic matter in the effluent and n is the number of cycles a day.

$$\mathrm{COR}_{\mathrm{SF}} = \frac{(C_{\mathrm{SAFL}} - C_{\mathrm{SF}}) \cdot V_A \cdot n}{V_u} \tag{1}$$

$$\mathrm{COR}_{\mathrm{ST}} = \frac{(C_{\mathrm{SAFL}} - C_{\mathrm{ST}}) \cdot V_A \cdot n}{V_u} \tag{2}$$

Results and Discussion

Average values of the monitored variables for preliminary tests are listed in Table 1, for the influent and effluent of the reactor, respectively. It can be seen in Table 1 that test (*IV*), in which the reactor operated with a renewal of 25% of the volume of reaction medium per cycle, was which presented the highest average value of efficiency of organic matter removal for filtered (95%) and unfiltered (90%) samples from the effluent, compared to the remaining tests. However, in all tests, efficiency of organic matter removal was calculated in relation to the concentration of organic matter in the influent and OLR was maintained at 12 gCOD l^{-1} day^{-1}. Efficiency of organic matter removal increased, mainly for filtered samples in the effluent, with decreasing renewed volume per cycle, although average values of the organic matter concentration in the effluent were very close. Therefore, comparison of the reactor performance during preliminary tests was done through the analysis of the concentrations of filtered and unfiltered organic matter rather than through the analysis of efficiency values.

In terms of concentration of filtered organic matter in the effluent, the four tests presented very close average values: 904, 952, 883 and 890 mgCOD l^{-1}, for tests (I), (II), (III) and (IV), respectively. However, in terms of concentration of unfiltered organic matter in the effluent, test (II) presented the lowest value: 1,151 mgCOD l^{-1} against 1,352, 1,533, and 1,592 mgCOD l^{-1}, for tests (I), (III) and (IV), respectively. With regard to the average TVA values in the effluent, these showed little variation, between 512 and 583 mgHAc l^{-1}. It was, hence established that the subsequent conditions for assessing the effect of different feeding times at different OLRs, in the treatment of cheese whey, would be carried out under conditions similar to those imposed on test (II), i.e., feed volume of cheese whey of 1.5 l in each cycle with maintenance of a residual volume of 1.0 l.

In Table 2, the average values of the monitored variables for the three investigated feeding times, for an OLR of 3 gCOD l^{-1} day^{-1}, are shown. In Figs. 2 and 3 are the values of concentration of organic matter and of total volatile acids, respectively, monitored during

Table 1 Average values of the monitored variables in the influent and in the effluent for the four preliminary tests (OLR of 12 gCOD l^{-1} day^{-1}).

Variable	Test			
	I[a]	II[b]	III[c]	IV[d]
Influent				
C_{SAFL} (mgCOD l^{-1})	4,010±71 (30)	5,732±303 (8)	8,116±100 (5)	16,060±200 (5)
TVA (mgHAc l^{-1})	204±15 (22)	273±12 (6)	440±14 (3)	812±32 (3)
BA (mgCaCO$_3$ l^{-1})	1,161±55 (22)	1,608±75 (6)	2,271±52 (3)	4,463±158 (3)
pH	7.8±0.1 (22)	7.8±0.1 (6)	7.6±0.1 (3)	7.6±0.2 (3)
TS (mg l^{-1})	5,539±101 (10)	7,587±549 (3)	9,890 (1)	21,667±1,956 (2)
TVS (mg l^{-1})	3,728±208 (10)	5,085±375 (3)	6,526 (1)	12,952±249 (2)
STS (mg l^{-1})	177±29 (10)	205±34 (3)	316 (1)	408±51 (2)
SSV (mg l^{-1})	135±26 (10)	142±43 (3)	204 (1)	230±48 (2)
Effluent				
C_{TS} (mgCOD l^{-1})	1,352±87 (11)	1,151±169 (7)	1,533±150 (5)	1,592±150 (5)
ε_{TS} (%)	66±2 (11)	80±3 (7)	81±2 (5)	90±1 (5)
C_{FS} (mgCOD l^{-1})	904±50 (11)	952±136 (7)	883±91 (5)	890±82 (5)
ε_{FS} (%)	78±1 (11)	83±2 (7)	89±1 (5)	95±1 (5)
TVA (mgHAc l^{-1})	512±31 (15)	568±86 (7)	583±60 (5)	550±46 (5)
BA (mgCaCO$_3$ l^{-1})	1,294±76 (15)	1,385±65 (7)	1,906±384 (5)	2,836±268 (5)
pH	7.3±0.1 (15)	7.3±0.2 (7)	7.4±0.2 (5)	7.8±0.2 (5)
TS (mg l^{-1})	2,791±76 (10)	3,286±280 (2)	3,566 (1)	5,407±912 (2)
TVS (mg l^{-1})	1,086±76 (10)	1,053±7 (2)	1,338 (1)	1,359±191 (2)
STS (mg l^{-1})	217±36 (10)	214±25 (2)	246 (1)	220±40 (2)
SSV (mg l^{-1})	175±45 (10)	165±38 (2)	176 (1)	142±8 (2)

Number in brackets indicates the number of samples used for averaging.

[a] Test I: feed volume of 2.5 l of cheese whey with no residual volume at a concentration of 4 gCOD l^{-1}, i.e., in this test no residual volume remained

[b] Test II: feed volume of 1.75 l of cheese whey with residual volume of 0.75 l and influent organic matter concentration of 5.70 gCOD l^{-1}

[c] Test III: feed volume of 1.25 l of cheese whey with residual volume of 1.25 l and influent organic matter concentration of 8 gCOD l^{-1}

[d] Test IV: feed volume of 0.63 l of cheese whey with residual volume of 1.87 l and influent organic matter concentration of 16 gCOD l^{-1} day^{-1}

the entire operational period for the OLR of 3 gCOD l^{-1} day^{-1}. The profiles of the main monitored variables run prior to and in the cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1} (day 25) at an OLR of 3 gCOD l^{-1} day^{-1}, are depicted in Figs. 4 and 5, respectively, for the 2-, 4-, and 6-h fed-batch operations.

For the OLR of 3 gCOD l^{-1} day^{-1}, increase in feeding time did not affect organic matter removal efficiency, which for the three tests remained around 98 and 94% for filtered and unfiltered samples of the effluent, respectively. Alkalinity supplementation to the influent could be optimized at 0.1 gNaHCO$_3$:1.0 gCOD$_{fed}$, which resulted in an average alkalinity of 172 mgCaCO$_3$ l^{-1} in the influent. TVA in the effluent remained around 56, 55, and 69 mgHAc l^{-1}, for feeding times of 2, 4, and 6 h, respectively, i.e., values inferior to the average value of 97 mgHAc l^{-1} observed in the influent, demonstrating the ability of the reactor to consume acids at this OLR.

Application of the shock load caused an increase in TVA in the effluent. The 2-h fed-batch operation presented the lowest TVA value referent to the shock: 741 mgHAc l^{-1}

Table 2 Average values of the monitored variables for the three investigated feeding times, at an OLR of 3 gCOD l^{-1} day^{-1}.

Variable	Influent	Effluent		
		t_A=2 h[a]	t_A=4 h[b]	t_A=6 h[c]
C_{TS} (mgCOD l^{-1})	1,686±30 (49)	99±29 (8)	109±12 (9)	107±13 (7)
ε_{TS} (%)	–	94±2 (8)	94±1 (9)	94±1 (7)
C_{FS} (mgCOD l^{-1})	–	43±16 (8)	42±8 (9)	41±7 (7)
ε_{FS} (%)	–	98±1 (8)	98±1 (9)	98±1 (7)
TVA (mgHAc l^{-1})	97±15 (38)	56±6 (6)	55±3 (9)	69±7 (10)
BA (mg$CaCO_3$ l^{-1})	172±18 (38)	253±37 (6)	273±31 (9)	296±13 (10)
pH	7.5±0.1 (38)	7.0±0.2 (6)	6.9±0.1 (9)	6.9±0.1 (10)
TS (mg l^{-1})	2,015±72 (17)	879±131 (5)	718±56 (6)	763±76 (4)
TVS (mg l^{-1})	1,568±64 (17)	352±91 (5)	345±76 (6)	338±101 (4)
STS (mg l^{-1})	78±18 (17)	101±31 (5)	73±32 (6)	66±28 (4)
SSV (mg l^{-1})	56±17 (17)	74±33 (5)	48±21 (6)	47±31 (4)
ROL_{FS} (gCOD l^{-1} day^{-1})	–	2.96 (1)	2.96 (1)	2.96 (1)
ROL_{TS} (gCOD l^{-1} day^{-1})	–	2.85 (1)	2.85 (1)	2.85 (1)
C_{CH4} (mmol l^{-1})	–	11.89 (1)	8.84 (1)	8.43 (1)

Number in brackets indicates the number of samples used for averaging

[a] Duration of the condition, 32 days

[b] Duration of the condition, 28 days

[c] Duration of the condition, 26 days

Biomass in the reactor (average value for this OLR): S_{TS}=1,784 mg g $foam^{-1}$; S_{TVS}=1,540 mg g $foam^{-1}$, S_{TS}=39.1 g l reaction $medium^{-1}$, S_{TVS}=33.8 g l reaction $medium^{-1}$, S_{TVS}/S_{TS}=0.86 gTVS gTS^{-1}

against 824 and 781 mgHAc l^{-1} for the 4- and 6-h fed-batch operations, respectively (Fig. 3). In the cycle subsequent to the shock load (day 26), the 2-h fed-batch operation also presented the lowest TVA in the effluent: 343 mgHAc l^{-1} against 582 and 468 mgHAc l^{-1} in the 4- and 6-h fed-batch operations, respectively (Fig. 3). Despite this increase the system presented improved ability to regain the process, corroborated by the values of the concentrations of organic matter and of total volatile acids, which 2 days after the shock load, showed to be very close to those prior to the shock, for the three investigated feeding times.

It can be seen from Fig. 4a and b that alteration in feeding time did not significantly affect the final concentration values of organic matter and of total volatile acids, respectively. However, the maximum values of these variables were affected during the

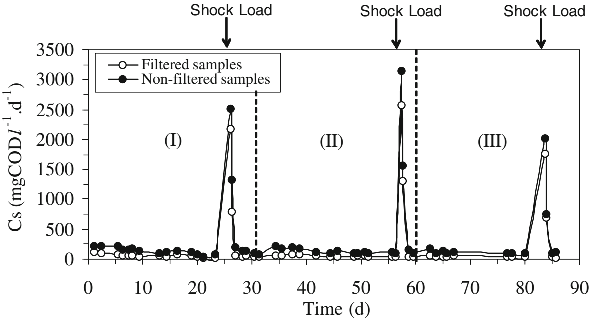


Fig. 2 Organic matter concentration in the effluent during fed-batch operation of 2, 4, and 6 h, for OLR=3 gCOD l^{-1} day^{-1} [Notation: *(I)* t_a=2 h; *(II)* t_a=4 h; *(III)* t_a=6 h]

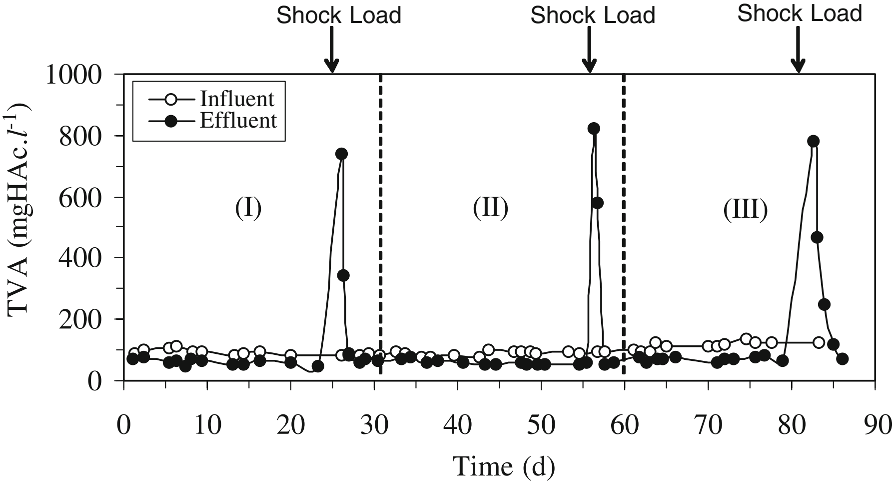


Fig. 3 Concentration of total volatile acids during fed-batch operation of 2, 4, and 6 h, for OLR=3 gCOD l^{-1} day^{-1} [Notation: *(I)* t_a=2 h; *(II)* t_a=4 h; *(III)* t_a=6 h]

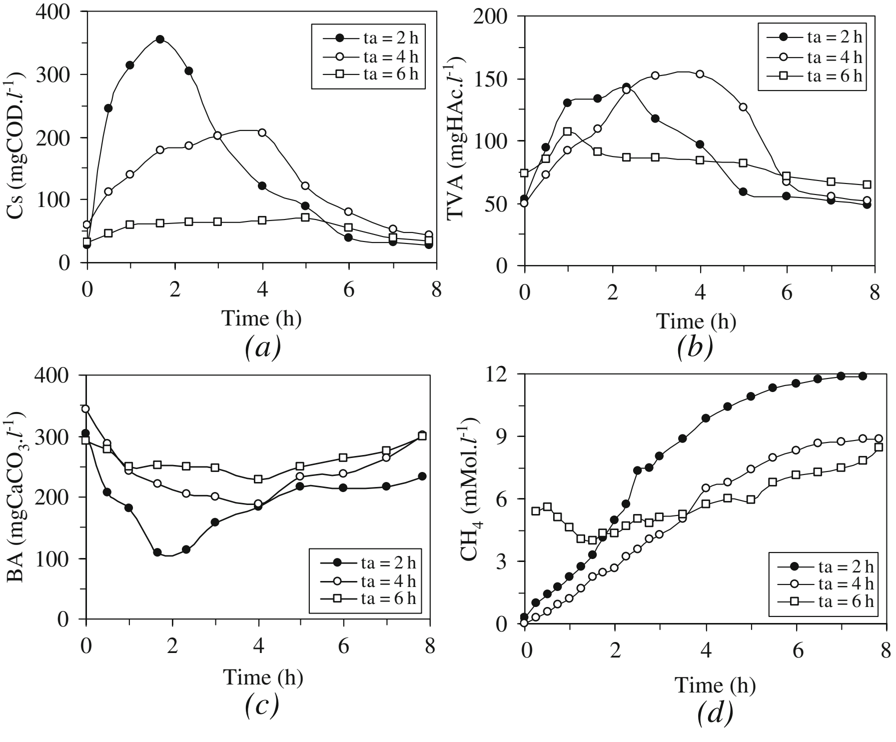


Fig. 4 Profiles of organic matter concentration **a**, total volatile acids **b** bicarbonate alkalinity **c** and methane **d** for the fed-batch operations of 2, 4, and 6 h, for OLR=3 gCOD l^{-1} day^{-1}

cycle. The 6-h fed-batch operation showed lower accumulation of acids. The acids with higher concentrations were: propionic acid, which attained 27.6 mg l^{-1} in 1 h and 31.4 mg l^{-1} in 4 h, for the 2- and 4-h fed-batch operations, respectively; and butyric acid, which attained 14.3 mg l^{-1} in 1.67 h and 10.0 mg l^{-1} in 4 h, for the 2- and 4-h fed-batch operations, respectively. Other acids, such as isobutyric, isovaleric, and caproic presented concentrations below 3.2 mg l^{-1} at all investigated feeding times. In the 6-h fed-batch operation no acids were detected, i.e., concentrations were below the detection limit of the method.

It can be seen in Fig. 4d that the concentration of methane at the end of the cycle for the 2-h fed-batch operation was 11.9 mmol l^{-1} and decreased to approximately 8.5 mmol l^{-1} in the 4- and 6-h fed-batch operations. An interesting observation was the higher methane concentration at the beginning of the cycle in the 6-h fed-batch operation (5.4 mmol l^{-1} day^{-1} in only 15 min) in relation to the 2- and 4-h operations. This behavior is likely due to the fact that in the 2- and 4-h fed-batch operations, where the organic matter is more readily available to the system in relation to the 6-h operation, acidogenesis (transformation of primary substrate to volatile acids) predominates more at the beginning of the cycle. The lower supply rate of organic matter to the system in the 6-h fed-batch operation also resulted in less accumulation of acids at the beginning of the cycle (Fig. 4b), with predominance of methanogenesis during the whole cycle. That is, the consumption of formed acids and consequent formation of methane occurred gradually and practically at the same rate during the entire cycle, whereby methane concentration oscillated less during the cycle in the 6-h fed-batch operation in relation to the 2- and 4-h operations, where the highest methane concentration occurred at the end of cycle. As seen for the organic matter removal efficiency, the values of removed organic load also showed to be independent of feeding time and remained around 2.96 and 2.85 gCOD l^{-1} day^{-1}, for filtered and unfiltered samples in the effluent, respectively.

The profiles run in the cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1} showed that the 2-h fed-batch operation was which provided the lowest TVA values (Fig. 5b) during the entire cycle, as well as values of organic matter concentration (Fig. 5a) very close to and slightly higher than those observed in the 6-h fed-batch operation. The acid encountered in the highest amount in the cycle subsequent to the shock load was butyric acid, which presented a maximum concentration of 143 mg l^{-1} at the beginning of the cycle, 245 mg l^{-1} after 0.5 h and 101 mg l^{-1} after 1.0 h, for the 2-, 4-, and 6-h fed-batch operations,

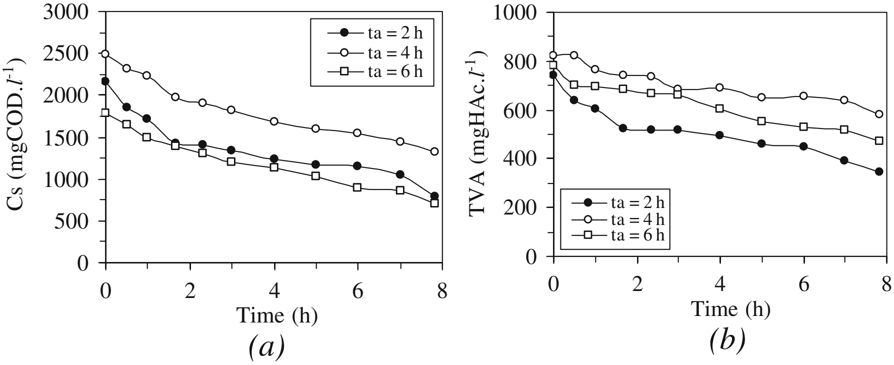


Fig. 5 Profiles of organic matter concentration **a** and of total volatile acids **b** for the fed-batch operations of 2, 4, and 6 h, for OLR=3 gCOD l^{-1} day^{-1} (cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1})

Table 3 Average values of the monitored variables for the three investigated feeding times, at an OLR of 6 gCOD l^{-1} day^{-1}.

Variable	Influent	Effluent		
		t_A=2 h[a]	t_A=4 h[b]	t_A=6 h[c]
C_{TS} (mgCOD l^{-1})	3,409±152 (37)	86±19 (8)	86±14 (7)	109±14 (6)
ε_{TS} (%)	–	98±1 (8)	98±1 (7)	97±1 (6)
C_{FS} (mgCOD l^{-1})	–	49±13 (8)	55±9 (7)	91±4 (6)
ε_{FS} (%)	–	99±1 (8)	98±1 (7)	97±1 (6)
TVA (mgHAc l^{-1})	166±11 (30)	120±14 (8)	124±7 (7)	113±7 (6)
BA (mgCaCO$_3$ l^{-1})	643±44 (30)	1,062±107 (8)	940±59 (7)	915±63 (6)
pH	7.5±0.2 (30)	7.2±0.1 (8)	6.9±0.2 (7)	7.1±0.2 (6)
TS (mg l^{-1})	3,993±77 (13)	1,810±287 (5)	1,444±14 (3)	1,744±96 (4)
TVS (mg l^{-1})	2,946±69 (13)	542±60 (5)	521±51 (3)	703±93 (4)
STS (mg l^{-1})	140±13 (13)	50±5 (5)	26±9 (3)	48±7 (4)
SSV (mg l^{-1})	105±16 (13)	34±9 (5)	16±2 (3)	28±9 (4)
ROL$_{FS}$ (gCOD l^{-1} day^{-1})	–	6.05 (1)	6.04 (1)	5.97 (1)
ROL$_{TS}$ (gCOD l^{-1} day^{-1})	–	5.98 (1)	5.98 (1)	5.94 (1)
C_{CH4} (mmol l^{-1})	–	11.46 (1)	16.80 (1)	17.68 (1)

Number in brackets indicates the number of samples used for averaging

[a] Duration of the condition, 30 days

[b] Duration of the condition, 20 days

[c] Duration of the condition, 27 days

Biomass in the reactor (average value for this OLR): S_{TS}=2,331 mg g $foam^{-1}$; S_{TVS}=2,015 mg g $foam^{-1}$; S_{TS}=50.3 g l reaction $medium^{-1}$, S_{TVS}=43.4 g l reaction $medium^{-1}$; S_{TVS}/S_{TS}=0.86 gTVS gTS^{-1}

respectively. Other acids like isobutyric, isovaleric, valeric, and caproic presented concentrations below 41.4 mg l^{-1} at all investigated feeding times.

Average values of the monitored variables for the three investigated feeding times are listed in Table 3 for an OLR of 6 gCOD l^{-1} day^{-1}. Concentrations of organic matter and of total volatile acids, monitored during the whole operation period, for an OLR of 6 gCOD l^{-1} day^{-1}, are shown in Figs. 6 and 7, respectively. Profiles of the main monitored variables, run prior to and in the cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1}, at an OLR of 6 gCOD l^{-1} day^{-1}, are shown in Figs. 8 and 9, respectively, for the 2-, 4-, and 6-h fed-batch operations.

Different to what was observed at an OLR of 3 gCOD l^{-1} day^{-1}, the increase in fill time, at an OLR of 6 gCOD l^{-1} day^{-1}, caused a slight drop in the organic matter removal

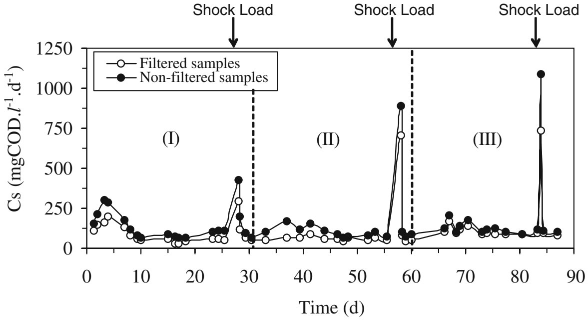
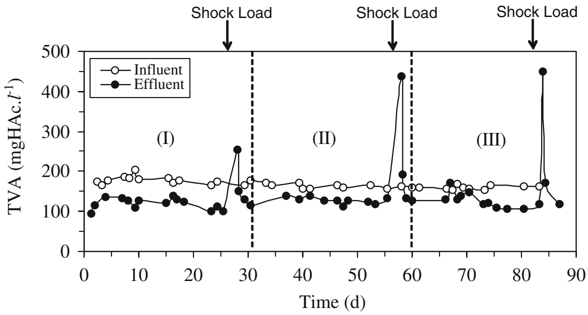
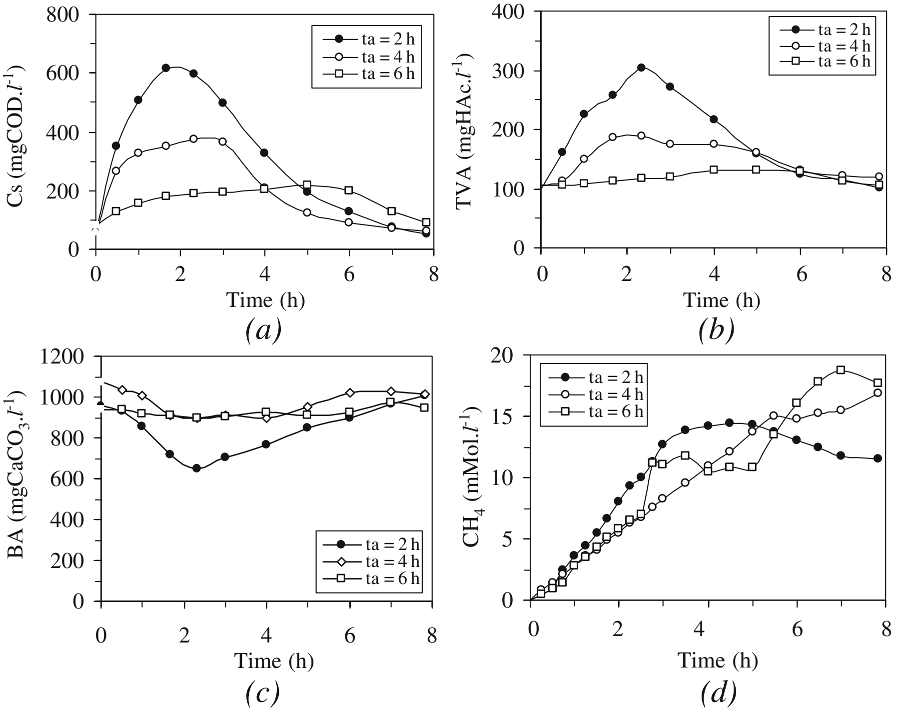


Fig. 6 Organic matter concentration in the effluent during fed-batch operation of 2, 4, and 6 h, for OLR=6 gCOD l^{-1} day^{-1} [Notation: *(I)* t_a=2 h; *(II)* t_a=4 h; *(III)* t_a=6 h]

Fig. 7 Concentration of total volatile acids during fed-batch operation of 2, 4, and 6 h, for OLR=6 gCOD l^{-1} day^{-1} [Notation: *(I)* t_a=2 h; *(II)* t_a=4 h; *(III)* t_a=6 h]



efficiency. Even so, high efficiency values were obtained, for both filtered samples (99, 98, and 97%, for feeding times of 2, 4, and 6 h, respectively) and unfiltered samples in the effluent (98% for feeding times of 2 and 4 h, and 97% for feeding time of 6 h). The TVA values in the effluent were 120, 124, and 113 mgHAc l^{-1} for the 2-, 4-, and 6-h fed-batch operations, respectively. These values were lower than the average TVA value in the influent (166 mgHAc l^{-1}), which makes obvious the ability of the reactor to consume acids. Supplementation of alkalinity could be optimized to 0.25 $gNaHCO_3$:1.0 $gCOD_{fed}$, sufficient to maintain pH close to neutral during the entire period.



(a) *(b)*

(c) *(d)*

Fig. 8 Profiles of organic matter concentration **a**, total volatile acids **b** bicarbonate alkalinity **c** and methane **d** for the fed-batch operations of 2, 4, and 6 h, for OLR=6 gCOD l^{-1} day^{-1}

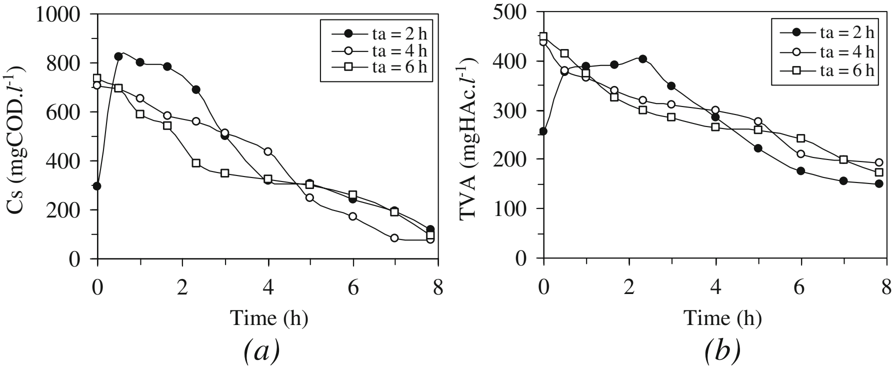


Fig. 9 Profiles of organic matter concentration **a** and of total volatile acids **b** for the fed-batch operations of 2, 4, and 6 h, for OLR=6 gCOD l^{-1} day^{-1} (cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1})

Operation with feeding time of 2 h, in addition to presenting the highest efficiency, also provided improved stability and lower susceptibility to shock loads in relation to the 4- and 6-h fed-batch operation. This fact can be confirmed by analyzing Figs. 6 and 7, which show lower concentrations of organic matter and of total volatile acids in the effluent of the cycle referent to the shock load for that feeding time. Organic matter concentrations, for filtered and unfiltered samples of the effluent, in the cycle referent to the shock load, were 295 and 426 mgCOD l^{-1}, respectively, for the 2-h fed-batch operation, 708 and 893 mgCOD l^{-1} for the 4-h fed-batch, and 736 and 1,087 mgCOD l^{-1} for the 6-h fed-batch operation (Fig. 6). The TVA values in the effluent of the cycle of the shock load, these were 256, 437, and 449 mgHAc l^{-1}, for the 2-, 4-, and 6-h fed-batch operations, respectively (Fig. 7). It can be seen in Table 3 that the 2-h fed-batch operation presented the highest average value of bicarbonate alkalinity in the effluent: 1,062 $mgCaCO_3$ l^{-1} against 940 and 915 $mgCaCO_3$ l^{-1} for the 4- and 6-h fed-batch operations, respectively. This is an important finding, since the decreased susceptibility to the shock load observed in the 2-h fed-batch operation might arise from the increased capacity to produce alkalinity under that condition. This alkalinity might have aided in neutralizing the acids formed during the shock load, providing improved process stability.

As observed for an OLR of 3 gCOD l^{-1} day^{-1}, alteration in fill time at an OLR of 6 gCOD l^{-1} day^{-1} did not significantly affect the final concentrations of organic matter and of total volatile acids (Fig. 8a and b). There was only a decrease in the maximum values of these variables during the cycle with increasing fill time. The acid with the highest concentrations encountered was propionic acid. This acid presented maximum concentration of 110 mg l^{-1} after 4 h, 73.6 mg l^{-1} after 3 h and 53.0 mg l^{-1} after 6 h, for the operations with feeding time of 2, 4, and 6 h, respectively. Thus, the increase in fill time likely minimized accumulation of hydrogen in the medium, providing increased control in the formation of propionic acid and therefore resulting in lower acid concentration for longer feeding times. Other acids like butyric, isobutyric, isovaleric, valeric, and caproic presented concentrations below 7.78 mg l^{-1} for the three investigated fill times. With regard to methane concentration at the end of the cycle, there was an increase with increasing feeding time: 11.4, 16.8 and 17.7 mmol l^{-1}, for the 2-, 4-, and 6-h fed-batch operations, respectively (Fig. 8d). Fill time, on its turn, did not cause significant variations in removed organic load (Table 3).

Profiles run in the cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1}, at an OLR of 6 gCOD l^{-1} day^{-1}, attest the lower susceptibility of the process to the shock load

Table 4 Average values of the monitored variables for the three investigated feeding times, at an OLR of 12 gCOD l^{-1} day^{-1}.

Variable	Influent	Effluent		
		t_A=2 h [(a)]	t_A=4 h [(b)]	t_A=6 h [(c)]
C_{TS} (mgCOD l^{-1})	6,689±159 (44)	289±51 (8)	516±63 (10)	816±82 (6)
ε_{TS} (%)	–	96±1 (8)	92±1 (10)	88±1 (6)
C_{FS} (mgCOD l^{-1})	–	220±44 (8)	336±37 (10)	484±54 (6)
ε_{FS} (%)	–	97±1 (8)	95±1 (10)	93±1 (6)
TVA (mgHAc l^{-1})	280±26 (28)	173±12 (6)	167±15 (10)	237±12 (5)
BA (mgCaCO$_3$ l^{-1})	959±95 (28)	1,389±54 (6)	1,227±43 (10)	1,220±39 (5)
pH	7.5±0.1 (28)	7.0±0.1 (6)	7.1±0.1 (10)	7.3±0.1 (5)
TS (mg l^{-1})	7,778±574 (10)	2,610±139 (2)	2,812±179 (3)	3,155±332 (4)
TVS (mg l^{-1})	5,864±469 (10)	822±37 (2)	1,172±140 (3)	1,282±176 (4)
STS (mg l^{-1})	259±26 (10)	110±3 (2)	186±20 (3)	235±31 (4)
SSV (mg l^{-1})	184±32 (10)	89±1 (2)	148±24 (3)	199±27 (4)
ROL$_{FS}$ (gCOD l^{-1} day^{-1})	–	11.64 (1)	11.43 (1)	11.17 (1)
ROL$_{TS}$ (gCOD l^{-1} day^{-1})	–	11.52 (1)	11.11 (1)	10.57 (1)
C_{CH4} (mmol l^{-1})	–	23.29 (1)	20.19 (1)	16.25 (1)

Number in brackets indicates the number of samples used for averaging

[a] Duration of the condition, 37 days

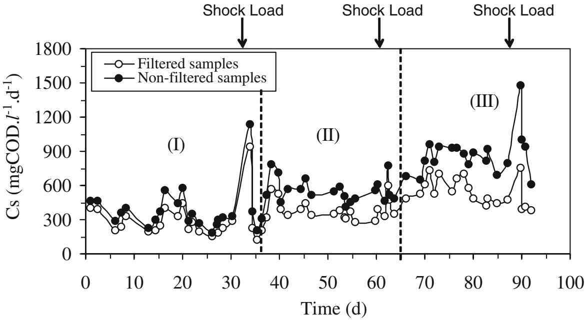
[b] Duration of the condition, 28 days

[c] Duration of the condition, 29 days

Biomass in the reactor (average value for this OLR): S_{TS}=2,285 mg g $foam^{-1}$; S_{TVS}=1,563 mg g $foam^{-1}$; S_{TS}=71.9 g l reaction $medium^{-1}$; S_{TVS}=49.2 g l reaction $medium^{-1}$; S_{TVS}/S_{TS}=0.68 gTVS gTS^{-1}

provided by the 2-h fed-batch operation in relation to the 4- and 6-h fed-batch operations (Figs. 9a and b). Despite the increase in concentration of organic matter and of total volatile acids in the first hours of the cycle, the 2-h fed-batch operation provided the lowest TVA values at the beginning and at the end of the cycle. In addition, the maximum acid concentration for the 2-h fed-batch operation was 403 mgHAc l^{-1} after 2.33 h, i.e., lower than the initial acid concentrations in the 4- and 6-h fed-batch operations, which were 437 and 449 mgHAc l^{-1}, respectively. For the three tests, the volatile acids encountered at higher amounts during the cycle subsequent to the shock load were butyric (maximum concentration of 18.5 mg l^{-1}) and isovaleric (maximum concentration of 29.3 mg l^{-1}). Valeric and isobutyric acid were encountered at lower amounts.

Fig. 10 Organic matter concentration in the effluent during fed-batch operation of 2, 4, and 6 h, for OLR=12 gCOD l^{-1} day^{-1} [Notation: *(I)* t_a=2 h; *(II)* t_a=4 h; *(III)* t_a=6 h]



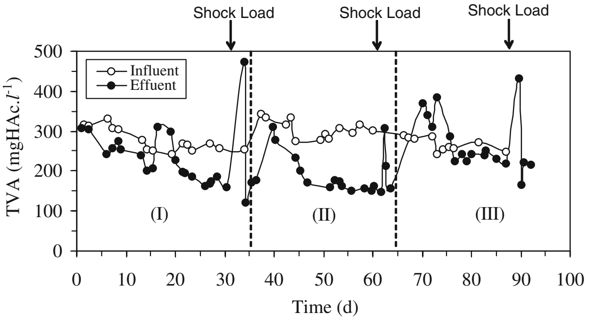


Fig. 11 Concentration of total volatile acids during fed-batch operation of 2, 4, and 6 h, for OLR=12 gCOD l^{-1} day^{-1} [Notation: *(I)* t_a=2 h; *(II)* t_a=4 h; *(III)* t_a=6 h]

Average values of the monitored variables for the three investigated feeding times, at an OLR of 12 gCOD l^{-1} day^{-1}, are listed in Table 4. In Figs. 10 and 11 are shown, respectively, concentrations of organic matter and of total volatile acids, monitored during the entire operational period at the aforementioned organic loading. Profiles of the main monitored variables, run prior to and in the cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1}, for an OLR of 12 gCOD l^{-1} day^{-1}, are depicted, respectively, in Figs. 12 and 13, for the 2-, 4- and 6-h fed-batch operations.

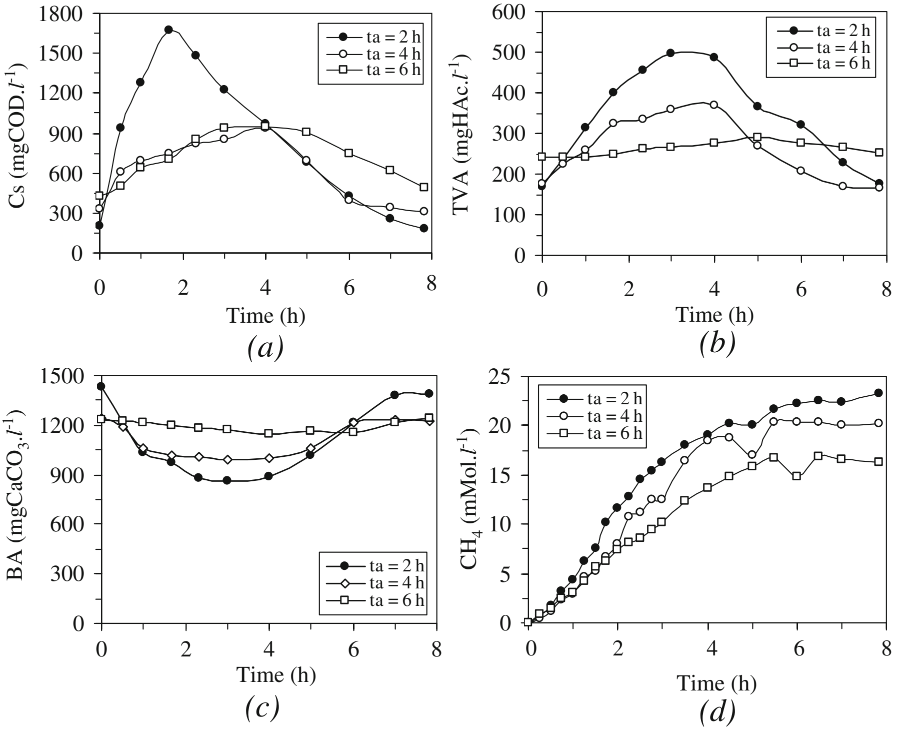


Fig. 12 Profiles of organic matter concentration **a** total volatile acids **b** bicarbonate alkalinity **c** and methane **d** for the fed-batch operations of 2, 4, and 6 h, for OLR=12 gCOD l^{-1} day^{-1}

Alteration in fill time, at an OLR of 12 gCOD l^{-1} day^{-1}, had a stronger effect on performance in relation to the previous load of 6 gCOD l^{-1} day^{-1}. The increase in feeding time resulted in a drop in the values of organic matter removal efficiency, which were around 97, 95, and 93% for filtered samples in the effluent, and 96, 92, and 88% for unfiltered samples in the effluent, for feeding times of 2, 4, and 6 h, respectively (Table 4). With regard to the average TVA value in the effluent, this remained around 170 mgHAc l^{-1} in the 2- and 4-h fed-batch operations and increased to 237 mgHAc l^{-1} in the 6-h fed-batch operation. However, the system remained stable during the entire period at this OLR. The highest acid concentration in the effluent of the reactor, in the 6-h fed-batch operation, occurred due to the short time available for the consumption of the formed acids, after conclusion of the filling (Fig. 12b).

The increase in fill time, on its turn, resulted in a decrease in the maximum value of TVA during the cycle. These were 498, 369, and 289 mgHAc l^{-1} for the 2-, 4-, and 6-h fed-batch operations, respectively (Fig. 12b). Consequently, the minimum value of bicarbonate alkalinity increased during the cycle as the feeding time increased: 859, 993, and 1,147 mg$CaCO_3$ l^{-1}, for the 2-, 4-, and 6-h fed-batch operations, respectively (Fig. 12-c). Supplementation of alkalinity to the influent could be optimized at 0.25 g$NaHCO_3$:$gCOD_{fed}$, which showed to be sufficient to buffer the medium and maintain pH close to neutral during the entire period. Acid and propionic acid were not detected under any condition. The main acids identified in the cycle were isovaleric, valeric, butyric, and isobutyric.

Analysis of Fig. 10 shows a significant increase in organic matter concentration in the effluent as fill time increases, corroborating the improved performance results for the 2-h fed-batch operation. Despite showing the second highest organic matter concentration (Fig. 10) and the highest TVA value in the effluent (Fig. 11) of the cycle referent to the shock load, the 2-h fed-batch operation was which provided the shortest recovery time of the process after the shock and was, therefore, once more, considered to be the condition that enabled reduced susceptibility of the reactor to shock loads. For the 4- and 6-h fed-batch operations, organic matter concentrations for filtered samples in the effluent, in the cycle subsequent to the shock load, were, respectively, 475 and 388 mgCOD l^{-1}, against 232 mgCOD l^{-1} obtained in the 2-h fed-batch operation (Fig. 10). With regard to the concentration of total volatile acids in the effluent of the cycle subsequent to the shock, the 2-h fed-batch operation also presented the best results: 122 mgHAc l^{-1}, against 214 and 164 mgHAc l^{-1}, obtained in the 4 and 6-h fed-batch operations, respectively (Fig. 11).

As observed during operation at OLR of 6 gCOD l^{-1} day^{-1}, the 2-h fed-batch operation also showed improved ability to produce bicarbonate alkalinity (Fig. 12-c), which might have aided in neutralizing the eventually formed acids during the shock load, providing improved process stability. The values of removed organic load help to complement the thesis of improved reactor performance for operation with feeding time of 2 h and confirm that this feeding time provides the best results not only in qualitative terms, i.e., in terms of organic matter concentration in the effluent, but also in terms of amount removed organic matter (Table 4). The 2-h fed-batch operation also presented the highest methane concentrations in the reactor atmosphere during the cycle, i.e., 23.3 mmol l^{-1}, against 20.2 and 16.3 mmol l^{-1} for the 4- and 6-h fed-batch operations, respectively (Fig. 12d).

Analysis of Figs. 13a and b shows that despite the higher variation in organic matter concentrations and total volatile acids in the cycle subsequent to the shock load, the 2-h fed-batch operation presented the lowest end values of these variables, corroborating the thesis of improved stability and less susceptibility to shock loads. It should be pointed out that when it is of interest to maintain low concentrations of organic matter during the cycle, either by applying a high organic load or due to the presence of a substance toxic to the

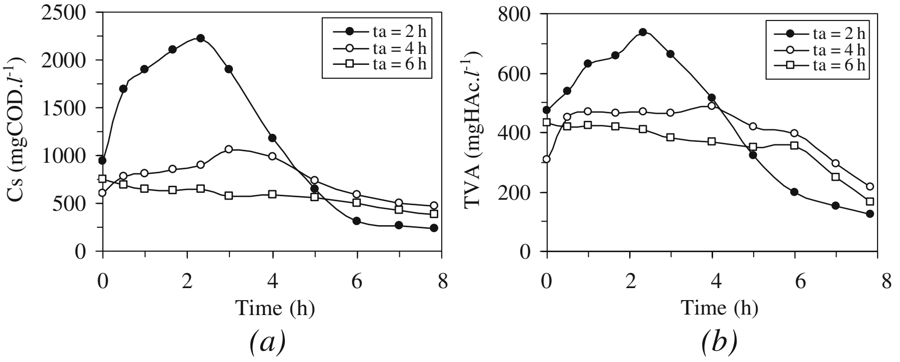


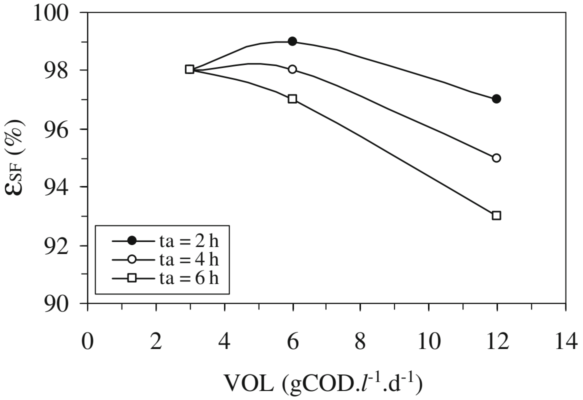
Fig. 13 Profiles of organic matter concentration **a** and of total volatile acids **b** for the fed-batch operations of 2, 4, and 6 h, for OLR=12 gCOD l^{-1} day^{-1} (cycle subsequent to the shock load of 24 gCOD l^{-1} day^{-1})

biomass in the wastewater, the 6-h fed-batch operation becomes interesting, as it causes smaller variation in organic matter concentration in the cycle subsequent to the shock load. However, when the accumulation of organic matter in the cycle is not significant to an extent to cause any inhibition to the process, and when it is of interest to accomplish a better quality effluent, the operation with feeding time of 2 h seems to be more convenient.

In Fig. 14, the efficiency of organic matter removal for filtered samples in the effluent as a function of OLR for the three feeding times used is shown. Operation with feeding time of 2 h was which presented the lowest sensitivity to elevation of organic load, confirming the best results obtained for this feeding time. Analysis of Fig. 14 allows seeing more clearly the stronger influence of fill time on reactor performance for higher OLRs. For the OLR of 3 gCOD l^{-1} day^{-1} reactor performance is independent of the feeding time used. For organic loads up to 6 gCOD l^{-1} day^{-1} increase in fill time did not cause significant variations in the values of filtered organic matter removal efficiency: 99 and 97% for the shortest and longest feeding time used, respectively.

These results are evidence for the flexibility of the reactor related to this operation strategy and make this operation mode a feasible alternative to the conventional batch operation, since in most cases in full scale reactors feeding time turns out to be considerable

Fig. 14 Efficiency of organic matter removal for filtered samples in the effluent as a function of OLR for different feeding times



in relation to total cycle time. For OLRs above 6 gCOD l^{-1} day^{-1} feeding time started having a more significant influence on filtered organic matter removal efficiency: 97 and 93% for the shortest and longest feeding time used, respectively, at the OLR of 12 gCOD l^{-1} day^{-1}. In general, considering the biodegradability and lack of alkalinity of cheese whey, the results obtained showed to be quite satisfactory, with values of efficiency of organic matter removal for filtered samples in the effluent above 92% in all tests.

Microbiological analyses of the polyurethane foam containing anaerobic immobilized biomass showed the existence of cells similar to bacilli and vibriones inside the support, as well as the presence of *Methanosaeta*-like and *Methanosarcina*-like morphologies, with equilibrium in the distribution of these two genera.

Conclusions

Results showed that the feeding time used had a stronger effect on reactor performance for higher OLRs. At an OLR of 3 gCOD l^{-1} day^{-1} the efficiency of organic matter removal showed to be independent of the feeding time used. For the operations with OLRs of 6 and 12 gCOD l^{-1} day^{-1} efficiency of organic matter removal dropped with increase in fill time; this drop was more significant for the operation at 12 gCOD l^{-1} day^{-1}. Shock loads caused an increase in TVA in the effluent; however, the alkalinity supplied to the influent was sufficient to maintain the pH of the medium close to neutral during the entire reactor operation. Contrary to the expected, utilization of longer feeding times did not result in minimization of the negative effects of the shock load. The 2-h fed-batch operation was that which provided lower concentration of volatile acids in the effluent and therefore improved stability of the reactor in the cycles subsequent to the shock, independent of the applied OLR, corroborating the best results obtained for this feeding time. It should, however, be mentioned that when it is of interest to maintain low concentrations of organic matter in the reactor, profiles showed that the 6-h fed-batch operation is still the most convenient. Under all conditions, methane, one of the end products of the process, was detected in the atmosphere of the reactor, proving that the reactor was not simply retaining or accumulating organic matter, but rather degrading it through anaerobic routes. In general, the reactor presented high efficiency and stability under all investigated conditions, evidencing its flexibility and potential application in the treatment of cheese whey under different operational conditions.

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