

Whey Treatment by AnSBBR with Circulation: Effects of Organic Loading, Shock Loads, and Alkalinity Supplementation

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Abstract The main objective of this work was to investigate the effect of volumetric loading rate (VLR), shock load, and alkalinity supplementation on the efficiency and stability of an Anaerobic Sequencing Batch Biofilm Reactor (AnSBBR) containing polyurethane foam cubes. Mixing in the reactor, which was kept at $30 \pm 1^\circ\text{C}$, occurred by recirculating the liquid phase. The reactor treated 2.5 l cheese whey in 8-h cycles, at concentrations of 1, 2, and 4 g COD l^{-1} , which corresponded to VLRs of 3, 6, and 12 g COD $\text{l}^{-1} \text{ day}^{-1}$, respectively. Application of single-cycle shock loads of 6, 12, and 24 g COD $\text{l}^{-1} \text{ day}^{-1}$ did not impair reactor performance. In addition, for VLRs of 3, 6, and 12 g COD $\text{l}^{-1} \text{ day}^{-1}$, alkalinity supplementation to the influent, at the end of each assay, could be reduced to 75, 50, and 50%, respectively, in relation to supplementation at the beginning of the assay. During reactor operation a viscous polymer-like material was formed between the polyurethane foam cubes, which increased at higher VLR. Finally, addition of salts to the influent improved reactor efficiency.

Keywords AnSBBR · Volumetric loading rate · Shock load · Cheese whey · Alkalinity supplementation

Abbreviations

AnSBBR anaerobic sequencing batch biofilm reactor
UASB up-flow anaerobic sludge blanket
COD chemical oxygen demand

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Notation**Symbols**

VLR	volumetric loading rate, g COD l ⁻¹ day ⁻¹
SOL	specific organic load, mg COD g TVS ⁻¹ day ⁻¹
C _{ST}	organic matter concentration in unfiltered effluent samples, mg COD l ⁻¹
C _{SF}	organic matter concentration in filtered effluent samples, mg COD l ⁻¹
BA	bicarbonate alkalinity, mg CaCO ₃ l ⁻¹
TVA	total volatile acids concentration, mg HAc l ⁻¹
TS	total solids concentration, mg l ⁻¹
TVS	total volatile solids concentration, mg l ⁻¹
TSS	total suspended solids concentration, mg l ⁻¹
VSS	volatile suspended solids concentration, mg l ⁻¹
VA _I	intermediate volatile acids concentration, mg HAc l ⁻¹
V _{CH4}	methane production, ml
% _{CH4}	methane percentage in the biogas, %
% _{CO2}	carbon dioxide percentage in the biogas, %
v _S	liquid phase recirculation velocity, cm s ⁻¹
Q	volumetric flow rate, l h ⁻¹
C _{SI}	organic matter concentration in unfiltered influent samples, mg COD l ⁻¹
C _S	organic matter concentration in unfiltered samples in the reactor along a cycle, mg COD l ⁻¹
V	reaction medium volume in the reactor, l
R _S	organic matter consumption rate, mg COD l ⁻¹ h ⁻¹
μ _S	specific organic matter consumption rate, mg COD g TVS h ⁻¹
C _{X-TV}	total volatile solids concentration relative to the reaction medium, g TVS l reaction medium ⁻¹
C _{X-TS}	total solids concentration relative to the reaction medium, g TS l reaction medium ⁻¹
C _{X-TV}	total volatile solids concentration relative to the immobilized biomass, mg TVS g foam ⁻¹
C _{X-TS}	total solids concentration relative to the immobilized biomass, mg TS g foam ⁻¹
μ	specific biomass growth rate, mg TVS g TVS ⁻¹ h ⁻¹
Y _{X/S}	organic matter–biomass conversion factor, mg TVS mg COD ⁻¹
M _{TVS}	mass of total volatile solids in the reactor, g TVS
μ _{max}	Maximum specific biomass growth rate, mg TVS g TVS h ⁻¹
k _S	Monod's kinetic constant, mg COD l ⁻¹
K	first order apparent kinetic constant, l g TVS ⁻¹ h ⁻¹
C _{SR}	residual filtered organic matter concentration, mg COD l ⁻¹
C _{SIO}	filtered organic matter concentration in the reactor at the beginning of the cycle, mg COD l ⁻¹
k ₁	first order apparent kinetic constant, h ⁻¹
T	cycle length, h
ROL _F	removed organic load for filtered effluent samples, g COD l ⁻¹ day ⁻¹
ROL _T	removed organic load for unfiltered effluent samples, g COD l ⁻¹ day ⁻¹
V _A	fed volume or renewed volume per cycle, l
t _c	cycle length, h
R ²	squared correlation coefficient
ε	organic matter removal efficiency, %

Introduction

The characteristics of wastewaters generated by dairy industries may vary widely, depending on the type of product, such as yoghurt, butter, milk, cheese, ice cream, etc. When product diversity is large, the effluent will usually consist of several streams with final organic matter concentration of 2 to 5 g COD l⁻¹. In the case of cheese as exclusive product, the presence of whey in the effluent may significantly increase organic matter concentration to as high as 70 g COD l⁻¹ [1].

However, most cheese producers, especially small- and medium-sized ones, do not have sufficient resources and means to invest in technologies for the reuse of the cheese whey generated in the process and end up disposing their effluents in the waterways without any kind of treatment. This disposal generates big environmental problems, as the polluting potential of cheese whey is about one hundred times higher than that of domestic wastewater [2].

Anaerobic treatment of whey is one of the most interesting alternatives to minimize this pollution problem. Different continuous anaerobic reactor configurations have been used in treating whey and other dairy effluents [3–6]; however, in the cheese industry, whey is usually disposed of intermittently, making batch treatment units more attractive.

Currently, the Anaerobic Sequencing Batch Reactor (ASBR) using granulated biomass is applied to the treatment of high strength wastewaters, such as beverage and dairy industry effluents, landfill leachate and intensive swine breeding wastes. Li and Mulligan [7] treating beer wastewater in a 2-l ASBR, containing granulated biomass, obtained COD reduction between 80 and 90% for organic loads between 2 and 10 kg COD/m³ day and below 80% for organic loads between 10 and 20 kg COD/m³ day with improved efficiency at 35°C. Ndegwa et al. [8] using a 12-l ASBR with sludge circulation (500 ml/min) to treat dilute swine slurries at temperatures of 20 and 35°C, on investigating COD reduction and biogas production interaction, obtained COD reduction of 90 and 84% for hydraulic times of 7.2 and 9.1 days, respectively.

Nowadays, application of the Anaerobic Sequencing Batch Biofilm Reactor (AnSBBR) using immobilized biomass on inert support is increasing. Mohan et al. [9] studied the bioaugmentation of an AnSBBR with alginate-immobilized sulfate reducing bacteria applied to sulfate-bearing treatment. The reactor with non-augmented biomass showed 35% of COD removal efficiency and 27% of sulfate reduction, and volatile acids accumulation occurred. After bioaugmentation the reactor performance improved attaining COD removal of 78% and sulfate reduction of 80% with concomitant increase in biogas and reduction in volatile acids. The microbial diversity distribution was also seen to improve with the presence of methanogenic, sulfate reducing and acetogenic bacteria. Mohan et al. [10] used an AnSBBR to treat hypersaline and low biodegradable wastewater to investigate the influence of recirculation on reactor performance. The reactor presented improvement in substrate removal efficiency with a maximum of 51%, and after introducing recirculation to the system, a biogas yield of 0.023 m/h, due to the improved mass transfer between the substrate present in the bulk liquid and the attached biofilm.

However, investigations focusing on AnSBBR for treating cheese whey and other dairy effluents are scarce in the literature, and still, little is known of the behavior of this type of system when submitted to increasing volumetric loading rate (VLR), which would be common during treatment, due to the frequent variations in effluent concentration observed in these industries. Moreover, due to its high biodegradability, high organic load, and lack of alkalinity, whey tends to acidify readily impairing maintenance of process stability at high VLRs. Hence, to maintain process stability, a system to control pH is necessary,

requiring, in most cases, addition of some external source of alkalinity, as bicarbonate, carbonate, or hydroxide [3]. In these cases, optimization of the amount of alkalinity added is of fundamental importance to minimize operation costs.

According to Demirel et al. [11], organic matter concentration and available alkalinity are the main factors affecting process stability, organic matter removal efficiency, and maximum volumetric loading rate that can be applied in the anaerobic treatment of whey. According to the authors, the effect of increasing volumetric loading rate on the stability of anaerobic reactors treating dairy effluents is one of the main parameters that currently deserve attention and require investigation.

Malaspina et al. [12], who used a downflow–up-flow hybrid anaerobic reactor with a useful volume of 107.5 l to treat cheese whey, observed that VLRs exceeding $10 \text{ g COD l}^{-1} \text{ day}^{-1}$ lead to a rapid increase in total volatile acids concentration and consequent drop in pH. From this point on, system recovery became impaired, and organic matter removal efficiency of 90% could only be maintained by addition of external alkalinity source and application of VLRs below $1 \text{ g COD l}^{-1} \text{ day}^{-1}$.

Ghaly et al. [6] used a two-stage mesophilic anaerobic digester to treat acid cheese whey. The volumetric loading rate applied was $4.8 \text{ g COD l}^{-1} \text{ day}^{-1}$. In one of the operation stages, there was no supplementation of NaHCO_3 in the second methanogenic reactor. At this stage, pH dropped to 3.3 in both reactors leading to system failure. Subsequently, pH was increased to 7.0 in the second reactor by adding NaHCO_3 in an attempt to recover the system. However, biomass inhibition was irreversible, and process recovery was not possible, as shown by the low organic matter removal efficiency observed. In another stage, where the system was reinoculated and pH controlled at 7.0 in the second methanogenic reactor by NaHCO_3 addition, the system maintained stability. At this stage, despite total volatile acids concentration of $1,640 \text{ mg HAc l}^{-1}$ in the second reactor, the biomass did not experience inhibition, as could be observed by maintenance of biogas production and both filtered and unfiltered organic matter removal efficiency which remained around 66.8 and 59.9%, respectively.

Ergüder et al. [5] used a lab-scale up-flow anaerobic sludge blanket (UASB) reactor to treat cheese whey and observed that organic matter removal efficiency remained practically the same, between 95.3 and 97%, for VLRs of 22.6 to $24.6 \text{ g COD l}^{-1} \text{ day}^{-1}$ and between 94.7 and 95.7%, for VLRs of 10.4 to $14.6 \text{ g COD l}^{-1} \text{ day}^{-1}$. The influent was supplemented with nutrients, trace metals, and NaHCO_3 ($6,000 \text{ mgCaCO}_3 \text{ l}^{-1}$). The volatile suspended solids concentration in the UASB was approximately 100 g VSS l^{-1} .

Mockaitis et al. [13] used an anaerobic sequencing batch reactor containing granular biomass for treating cheese whey. The mechanically stirred reactor, with useful volume of 5 l treated 2 l whey in 8-h cycles at VLRs of 0.59, 1.15, 2.50, and $4.79 \text{ g COD l}^{-1} \text{ day}^{-1}$, which corresponded to specific organic loads (SOL) of 42.4, 44.3, 76.4, and $137.8 \text{ mg COD g TVS}^{-1} \text{ day}^{-1}$. The reactor showed to be stable and efficient at all investigated VLRs and presented organic matter removal efficiency close to 90% for filtered samples. NaHCO_3 was added to the influent at ratios of 0.50, 0.50, 0.25, and $0.25 \text{ g NaHCO}_3 \text{ g COD}^{-1}$, for VLRs of 0.59, 1.15, 2.50, and $4.79 \text{ g COD l}^{-1} \text{ day}^{-1}$, respectively. At VLR of $9.60 \text{ g COD l}^{-1} \text{ day}^{-1}$ the reactor showed loss in stability and drop in organic matter removal efficiency. Moreover, at VLRs of 2.50, 4.79, and $9.60 \text{ g COD l}^{-1} \text{ day}^{-1}$, flotation of biomass occurred, likely due to release of carbonic gas, as the amount of floated biomass increased with increasing alkalinity supplementation.

Within this context, the main objective of the current work was to assess the effect of volumetric loading rate, shock load, and alkalinity supplementation on the efficiency and stability of an AnSBBR with liquid-phase recirculation treating cheese whey, and in this way, collaborate with correct operation of this reactor.

Materials and Methods

Experimental Set-up

The reactor shown in Fig. 1 consisted of an acrylic cylindrical column with height of 540 mm, external diameter of 100 mm, and wall thickness of 3.5 mm and was provided with an automatic unit for feeding, discharge, and recirculation of the liquid phase. The recirculation unit comprised: (1) a side reservoir with total capacity of 2.1 l, consisting of a cylindrical acrylic container with height of 300 mm, external diameter of 100 mm, and wall thickness of 3.5 mm; and (2) a peristaltic pump with maximum capacity of 30 l h^{-1} . A 100-ml measuring cylinder was attached to the recirculation system for flow rate measurements.

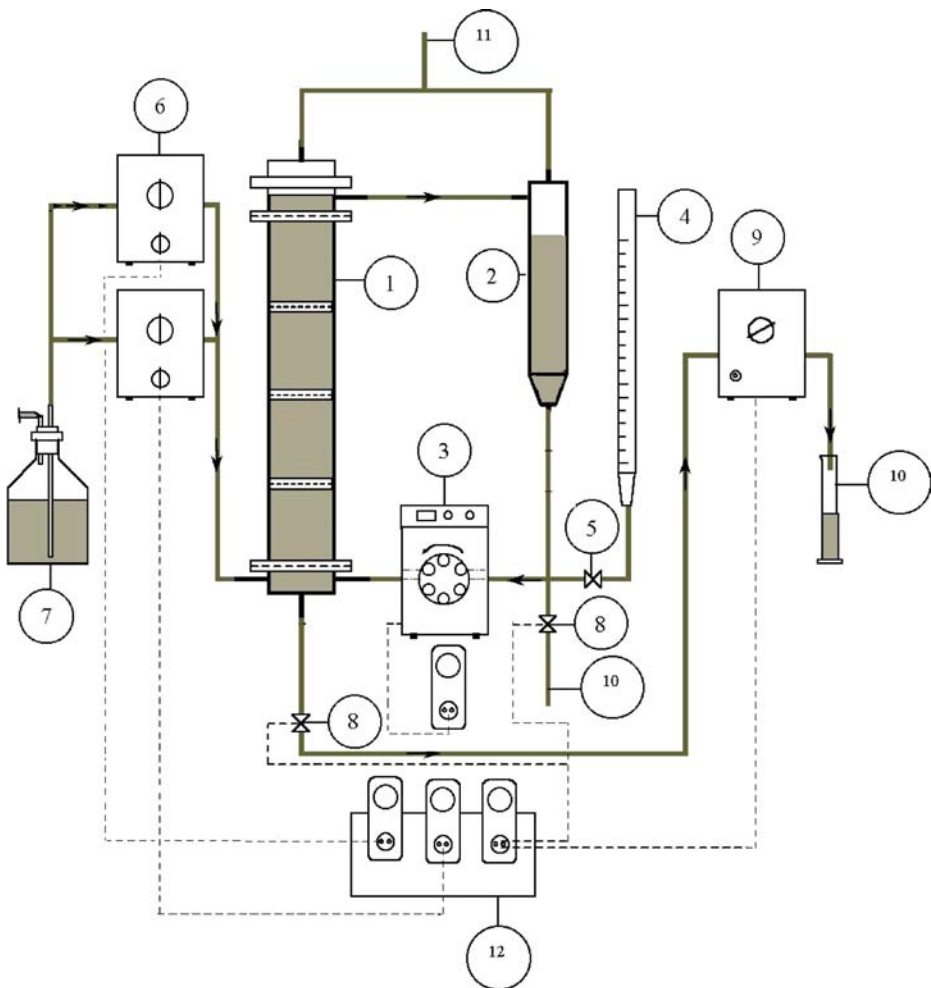


Fig. 1 Schematic representation of the system used for treating cheese whey. 1 Reactor containing immobilized biomass, 2 circulation reservoir, 3 circulation pump, 4 flow rate meter, 5 valve, 6 feed pump, 7 synthetic wastewater reservoir, 8 discharge valves, 9 discharge pump, 10 effluent outlet, 11 biogas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

Feeding and discharge were performed using diaphragm pumps equipped with automatic timers. The chamber in which the reactor remained was kept at $30 \pm 1^\circ\text{C}$ by a heating system composed of resistances and fans, and a temperature sensor and controller.

The inert support containing the immobilized biomass 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, this way, formation of preferential routes, and at the upper part of the reactor, a 40-mm region functioned as a biogas collector (CH_4 and CO_2).

Inoculum

The inoculum used in all experiments came from an up-flow anaerobic sludge blanket (UASB) reactor treating wastewater from a poultry slaughterhouse. Immobilization procedure, after Zaiat et al. [14], consisted of crushing the sludge through a 0.5-mm mesh nylon sieve, completely immersing the foam with the obtained suspension followed by intense homogenization, and 2-h rest. Poorly adhered solids were washed off and the medium drained. This inoculum presented total volatile solids (TVS) and total solids (TS) of 51 and 62 g/l, respectively.

Inert Support Containing the Biomass

The inert support used consisted of 1-cm polyurethane foam cubes with apparent density of 23 kg/m³ and porosity of 95%. One of the main advantages of using polyurethane foam is related to its high porosity, which allows immobilization of a significant amount of biomass that does not become detached during the charge, discharge, and reaction steps on recirculating of the liquid phase by the reactor [15].

Wastewater

Wastewater was prepared by dissolving dehydrated cheese whey in tap water; experimentally, 1 g dehydrated whey corresponds to approximately 1 g COD. The dehydrated cheese whey consisted of proteins (11%), carbohydrates (70%), lipids (1%), mineral salts (7%), and moisture (2%).

General Experimental Procedure

The reactor was operated in 8-h sequencing batch cycles, i.e., three cycles a day. At the beginning of each cycle, the reactor was fed with 2.5 l wastewater in 10 min. Next, recirculation of the medium was initiated at a velocity of 0.19 cm s^{-1} [16] for 460 min. After this period, at the end of the cycle, recirculation was interrupted, and the reactor was discharged in 10 min. The reaction medium volume in the reactor was 2.5 l, equal to the volume of wastewater treated per cycle.

Experimental Procedure Regarding the Effects of Salts Supplementation and Liquid Phase Recirculation Velocity

Before investigating the effect of volumetric loading rate on reactor stability and efficiency, some preliminary assays were performed (*Assays A, B, C and D*). These assays presented

some particularities in terms of reactor operation, namely: (Assay A) supplementation of salts to the influent and utilization of liquid phase recirculation velocity (v_s) lower (0.14 cm s^{-1}) than that of the remaining assays B, C, and D (0.19 cm s^{-1}); (Assay B) no supplementation of salts to the influent, i.e., influent consisted only of cheese whey and sodium bicarbonate; (Assay C) supplementation of synthetic wastewater to the influent; and (Assay D) supplementation of salts to the influent.

Assay A was carried out to test whether increase in residence time of the wastewater in the bed would increase organic matter removal, without significant decrease in external mass transfer rate. The objective of assays B, C, and D was to determine whether salt and wastewater supplementation to the influent would be necessary or not. In all preliminary assays, the reactor was operated with influent cheese whey concentration (C_{SI}) of approximately 4 g COD l^{-1} (which corresponded to a volumetric loading rate of approximately $12 \text{ g COD l}^{-1} \text{ day}^{-1}$) and alkalinity supplementation to the influent of $0.5 \text{ g NaHCO}_3 \text{ g COD}^{-1}$.

Tables 1 and 2 show the main operation characteristics and wastewater composition used, respectively, for the four assays performed. From the results of these assays, it was established that investigating the effect of volumetric loading rate on reactor performance would be carried out according to the conditions of Assay D, as this assay showed to be the most advantageous of the four.

Experimental Procedure Regarding the Effects of Volumetric Loading Rate

The investigation of the effect of volumetric loading rate on process efficiency and stability was carried out by operating the reactor at increasing influent concentrations: 1, 2, and 4 g COD l^{-1} , which corresponded to VLRs of 3, 6, and $12 \text{ g COD l}^{-1} \text{ day}^{-1}$, respectively. Optimization of alkalinity supplementation was carried out as follows: for each volumetric loading, rate reactor operation was started with NaHCO_3 supplementation at a 1:1 ratio of $\text{g NaHCO}_3 \text{ g COD}^{-1}$, and as soon as stability was attained of filtered organic matter concentration, total volatile acids concentration, and bicarbonate alkalinity, this ratio was reduced so as to determine the minimum amount of alkalinity required to confer system stability.

After reaching stability and having established the optimum amount of alkalinity, profiles were taken of the following monitored variables: C_{SF} , BA, TVA, V_{CH_4} , $\%CH_4$, $\%CO_2$, AV_I , and pH. These profiles allowed better understanding of the degradation routes along cycles and also enabled obtaining organic matter degradation kinetic parameters, by fitting a first order kinetic model to the experimental filtered organic matter concentration data.

Table 1 Main operation characteristics of the preliminary assays^a.

Operation characteristic	Assay			
	<i>A</i> ⁽⁸⁾	<i>B</i> ⁽³⁾	<i>C</i> ⁽¹¹⁾	<i>D</i> ⁽¹⁰⁾
v_s (cm s^{-1})	0.14	0.19	0.19	0.19
Supplementation of salts	Yes	no	No	Yes
Supplementation of synthetic wastewater	No	no	Yes	No

^a Numbers in parentheses denote the number of days of each assay.

Table 2 Composition of the synthetic wastewater used in Assays A, B, C, and D.

Assays A and D		Assay B		Assay C	
Compound	Concentration (mg l ⁻¹)	Compound	Concentration (mg l ⁻¹)	Compound	Concentration (mg l ⁻¹)
Cheese whey	4,000	Cheese whey	4,000	Cheese whey	4,000
NiSO ₄ ·6H ₂ O	2.00	–	–	Sucrose	85.2
FeSO ₄ ·7H ₂ O	10.0	–	–	Starch	278
FeCl ₃ ·6H ₂ O	1.00	–	–	Cellulose	82.8
CaCl ₂ ·2H ₂ O	94.0	–	–	Meat extract	507
CoCl ₂ ·6H ₂ O	0.16	–	–	Soy oil	124
SeO ₂	0.14	–	–	NaCl	609
KH ₂ PO ₄	170	–	–	MgCl ₂ ·6H ₂ O	16.8
K ₂ HPO ₄	43.4	–	–	CaCl ₂ ·2H ₂ O	10.8
Na ₂ HPO ₄	66.8	–	–	–	–
NaHCO ₃	2,000	NaHCO ₃	2,000	NaHCO ₃	2,000

Experimental Procedure Regarding the Effects of Shock Load

After attaining stability, the reactor was submitted to one-cycle shock loads, by doubling the influent concentration used. That is, in the first operation condition where the applied volumetric loading rate was 3 g COD l⁻¹ day⁻¹, a shock load of 6 g COD l⁻¹ day⁻¹ was applied, and in the cycle following the shock load, the volumetric loading rate was set to 3 g COD l⁻¹ day⁻¹ again. For the second and third operation condition where the applied volumetric loading rate was 6 and 12 g COD l⁻¹ day⁻¹, shock loads were 12 and 24 g COD l⁻¹ day⁻¹, respectively. With the objective to assess the effect of shock loads on reactor performance and its behavior after this perturbation, profiles (of C_{SF}, BA, and TVA) were also taken in the cycle subsequent to the shock load.

For each operation condition, the system was carefully monitored during the cycles subsequent to shock load application to verify if the reactor would maintain its efficiency and stability after the perturbation and the recovery time and process regain in case there were any kind of system destabilization. At stable condition, the new operation condition was initiated, i.e., influent concentration was increased definitively for longer periods.

Analytical Methods

The operating variables monitored according to Standard Methods for Examination of Water and Wastewater [17] were as follows: substrate concentration (measured as COD) for unfiltered (C_{ST}) and filtered samples (C_{SF}), bicarbonate alkalinity (BA), total volatile acids (TVA), total solids (TS), total volatile solids (TVS), total suspended solids (TSS), and volatile suspended solids (VSS). 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 injection volume was 1 µl. Methane production (V_{CH4}) and composition of the biogas (%CH₄–%CO₂) generated by anaerobic degradation were analyzed, respectively, by a gasmeter with NaOH solution (50 g l⁻¹), to promote CO₂ absorption, and by gas chromatography using a Hewlett Packard® 6890 gas chromatograph equipped with a thermal conductivity detector. The sample volume was 1 ml, and the drag

gas was hydrogen, at a flow rate of 50 ml min⁻¹. The column, injector, and detector temperatures were 35, 60, and 160°C, respectively.

Microbiology Analysis

At the end of each operation condition, bioparticle samples were withdrawn 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 solids relative to the immobilized biomass in the reactor, i.e., analysis of concentrations of total solids (C_{X-TS}) and total volatile solids (C_{X-TVS}) in the reactor, enabling this way to estimate the biomass concentration in the reactor as total volatile solids. It should be pointed out that the salt concentration values in Assay D (Table 2) are based on a cheese whey concentration of 4 g COD l⁻¹. As cheese whey concentration in the wastewater varied along the investigated conditions, salt concentration also varied proportionally.

Performance Parameters Calculation

For all operating conditions studied, removed organic loading (ROL) was calculated for filtered and unfiltered effluent samples. ROL can be defined as the amount of organic matter removed by the reactor per time unit and per reactor medium volume, for example, g DQO l⁻¹ day⁻¹. For batch reactors, organic loading removal for filtered (ROL_F) and unfiltered (ROL_T) effluent samples was calculated using Eqs. 1 and 2, respectively, where C_{SI} is the organic matter concentration in the influent, C_{SF} is the filtered organic matter concentration in the effluent, C_{ST} is the unfiltered organic matter concentration in the effluent, V_A is the volume of wastewater fed per cycle, V is the volume of wastewater in the reactor, and t_c is the cycle time.

$$ROL_F = \frac{(C_{SI} - C_{SF}) \cdot V_A}{V \cdot t_c} \quad (1)$$

$$ROL_T = \frac{(C_{SI} - C_{ST}) \cdot V_A}{V \cdot t_c} \quad (2)$$

The volumetric loading rate, defined as the amount of organic matter applied to the reactor per time unit and per reactor medium volume, for example, g DQO l⁻¹ day⁻¹, was calculated using Eq. 3.

$$VLR = \frac{C_{SI} \cdot V_A}{V \cdot t_c} \quad (3)$$

Finally, the specific organic load (SOL) can be calculated by Eq. 4, where C_{X-TVS} is total volatile solids concentration relative to the immobilized biomass in the reactor, which gives an estimation of the biomass concentration in the reactor. SOL may be defined as the amount of organic matter applied to the reactor per time unit and amount of biomass in the reactor (mg COD g TVS day⁻¹):

$$SOL = \frac{C_{SI} \cdot V_A}{C_{X-TVS} \cdot V \cdot t_c} \quad (4)$$

Kinetic Model Formulation and Fitting

Considering a completely stirred and isothermal reactor and admitting the occurrence of biological reaction, the following mass balance results:

$$\frac{dC_{SF}}{dt} = \frac{Q \cdot (C_{SI} - C_{SF})}{V} - R_S \quad (5)$$

In Eq. 5, C_{SF} is the filtered organic matter concentration (mg COD l^{-1}), Q the volumetric flow rate (l h^{-1}), C_{SI} the organic matter concentration in the influent (mg COD l^{-1}), V the reaction medium volume in the reactor (l), and R_S the organic matter consumption rate (mg COD $\text{l}^{-1} \text{h}^{-1}$).

As for batch operation, $Q=0$, Eq. 5 results in Eq. 6:

$$\frac{dC_{SF}}{dt} = -R_S \quad (6)$$

The kinetic model used (Eq. 7) was deduced from the model proposed by Monod, assuming biomass concentration in the reactor remains practically constant (accepted hypothesis for anaerobic metabolism in short-term experiments):

$$R_S = \mu_S \cdot C_{X\text{-TVS}} = \frac{\mu}{Y_{X/S}} \cdot \left(\frac{M_{\text{TVS}}}{V} \right) = \frac{1}{Y_{X/S}} \cdot \frac{\mu_{\max} \cdot C_{SF}}{k_S + C_{SF}} \cdot \left(\frac{M_{\text{TVS}}}{V} \right) \quad (7)$$

In Eq. 7, μ_S is the specific organic matter consumption rate (mg COD g TVS $^{-1} \text{h}^{-1}$), $C_{X\text{-TVS}}$ is the total volatile solids concentration in the reactor (g TVS l^{-1}), μ is the specific biomass growth rate (mg TVS g TVS $^{-1} \text{h}^{-1}$), $Y_{X/S}$ is the organic matter–biomass conversion factor (mg TVS mg COD $^{-1}$), M_{TVS} the mass of total volatile solids in the reactor (g TVS), μ_{\max} the maximum specific biomass growth rate (mg TVS g TVS $^{-1} \text{h}^{-1}$), and k_S is Monod's kinetic constant (mg COD l^{-1}).

Considering the reaction as first order, Eq. 8 results, where k is the first order apparent kinetic constant ($= \mu_{\max} \cdot Y_{X/S}^{-1} \cdot k_S^{-1}$), ($\text{l g TVS}^{-1} \text{h}^{-1}$):

$$\mu_S \cdot C_{X\text{-TVS}} = k \cdot \left(\frac{M_{\text{TVS}}}{V} \right) \cdot C_{SF} \quad (8)$$

Considering the existence of a residual organic matter concentration value, where the organic matter consumption rate is practically zero, the kinetic model was modified to include this residual concentration (Eq. 9), where C_{SR} is the residual filtered organic matter concentration (mg COD l^{-1}):

$$\mu_S \cdot C_{X\text{-TVS}} = k \cdot \left(\frac{M_{\text{TVS}}}{V} \right) \cdot (C_{SF} - C_{SR}) \quad (9)$$

Fitting of the model to the experimental profile data of filtered organic matter concentration was accomplished by means of the Levenberg–Maquardt method (Microcal Origin 6.1©). The process model is given by Eq. 10, where C_{SF} is the filtered organic matter concentration in the reactor, C_{SIO} is the organic matter concentration in the reactor at the beginning of the cycle, k_1 is the first-order apparent kinetic constant ($= k M_{\text{TVS}} V^{-1}$), t is the cycle length, and C_{SR} is the residual filtered organic matter concentration. It should be mentioned that the proposed kinetic model was formulated considering a homogeneous reactor, i.e., parameter k_1 obtained by the model is an apparent kinetic constant, which

includes not only the intrinsic kinetics of biochemical reaction, but also the bioparticle internal and external mass transfer phenomena.

$$C_{SF} = (C_{SIO} - C_{SR}) \cdot e^{-k_1 \cdot t} + C_{SR} \quad (10)$$

Results and Discussion

Effects of Supplementation of Salts and Liquid Phase Recirculation Velocity

For comparison's sake, Table 3 presents the average values of the monitored variables for the four preliminary assays performed. The lowest organic matter removal efficiency values were obtained in Assay B, showing that the lack of salts in the medium reduced reactor performance. Supplementation of synthetic wastewater to the influent, performed in Assay C, increased reactor efficiency in relation to Assay B. However, replacing supplementation of synthetic wastewater in the influent (Assay C) by supplementation of salts (Assay D) resulted in increase in organic matter removal efficiency, especially for filtered effluent samples.

Of all assays, Assay D, where the reactor was operated with supplementation of salts in the influent and with liquid phase recirculation velocity of 0.19 cm s^{-1} , was that which showed the highest average value of organic matter removal efficiency for filtered effluent samples, i.e., 78%, against 76, 72, and 73%, in Assays A, B, and C, respectively. As to organic matter removal efficiency for unfiltered effluent samples, Assay D presented a slightly lower average value (70%) than Assay A (71%). As to bicarbonate alkalinity, Assay D showed highest production compared to the remaining, i.e., $1,365 \text{ mg Ca CO}_3 \text{ l}^{-1}$ against 1,317, 937, and 1,052 $\text{mg CaCO}_3 \text{ l}^{-1}$, in Assays A, B, and C, respectively. As to total volatile acids concentration in the effluent, average values ranged from 509 to 610 mg HAc l^{-1} for all assays, with the lowest value in Assay D (509 mg HAc l^{-1}). Therefore, it was decided to employ the conditions of Assay D, i.e., liquid phase recirculation velocity of 0.19 cm s^{-1} and supplementation of salts to the influent, to investigate the effect of

Table 3 Average values of monitored variables in the preliminary assays^a.

Variable	Influent ^b	Effluent			
		Assay A	Assay B	Assay C	Assay D
C_{ST} (mg COD l^{-1})	$4,534 \pm 533^{(20)}$	$1,153 \pm 50^{(4)}$	$1,464 \pm 54^{(3)}$	$1,647 \pm 161^{(5)}$	$1,227 \pm 79^{(8)}$
ε_{ST} (%)	–	$71 \pm 1^{(4)}$	$64 \pm 1^{(3)}$	$68 \pm 3^{(5)}$	$70 \pm 2^{(8)}$
C_{SF} (mg COD l^{-1})	–	$996 \pm 47^{(4)}$	$1,138 \pm 19^{(3)}$	$1,362 \pm 83^{(5)}$	$915 \pm 53^{(8)}$
ε_{SF} (%)	–	$76 \pm 1^{(4)}$	$72 \pm 1^{(3)}$	$73 \pm 2^{(5)}$	$78 \pm 1^{(8)}$
TVA (mg HAc l^{-1})	$184 \pm 21^{(19)}$	$532 \pm 38^{(4)}$	$610 \pm 19^{(3)}$	$535 \pm 70^{(5)}$	$509 \pm 32^{(8)}$
BA (mg $\text{CaCO}_3 \text{ l}^{-1}$)	$1,178 \pm 38^{(19)}$	$1,317 \pm 62^{(4)}$	$937 \pm 68^{(3)}$	$1,052 \pm 82^{(5)}$	$1,365 \pm 55^{(8)}$
pH	$8.0 \pm 0.1^{(19)}$	$7.2 \pm 0.1^{(4)}$	$7.2 \pm 0.1^{(3)}$	$7.1 \pm 0.1^{(5)}$	$7.3 \pm 0.2^{(8)}$
TS (mg l^{-1})	$5,975 \pm 1166^{(9)}$	$2,870^{(1)}$	$2,575 \pm 52^{(2)}$	$3,279 \pm 166^{(3)}$	$2,783 \pm 103^{(4)}$
TVS (mg l^{-1})	$4,154 \pm 833^{(9)}$	$1,056^{(1)}$	$1,126 \pm 99^{(2)}$	$1,224 \pm 87^{(3)}$	$1,126 \pm 70^{(4)}$
TSS (mg l^{-1})	$168 \pm 36^{(9)}$	$176^{(1)}$	$197 \pm 89^{(2)}$	$177 \pm 33^{(3)}$	$205 \pm 19^{(4)}$
VSS (mg l^{-1})	$132 \pm 43^{(9)}$	$134^{(1)}$	$157 \pm 66^{(2)}$	$146 \pm 32^{(3)}$	$169 \pm 13^{(4)}$

^a Numbers in parentheses refer to the number of samples used for averaging.

^b Average of the four assays performed

volumetric loading rate, shock load, and alkalinity supplementation on reactor stability and efficiency.

Effects of Volumetric Loading Rate

Tables 4 and 5 show the average values of the monitored variables in the influent and effluent of the reactor at the three investigated conditions, respectively. Increase in volumetric loading rate resulted in a drop in organic matter removal efficiency. At VLRs of 3, 6, and 12 g COD l⁻¹ day⁻¹, average organic matter removal efficiency values were 96, 83, and 78%, for filtered samples, and 91, 77, and 66%, for unfiltered effluent samples, respectively. Figure 2 shows the decrease in organic matter removal efficiency with increasing volumetric loading rate.

At all conditions, total solid concentration and total volatile solids in the effluent showed to be inferior to those in the influent (Tables 4 and 5), evidencing conversion of total solids and total volatile solids by the system. However, total suspended solids and suspended volatile solids in the effluent were seen to be superior to those in the influent, which is likely due to the formation of polymer-like viscous material, probably of microbiological origin, between the polyurethane foam cubes in the reactor, which was discharged with the effluent during reactor discharge. Formation of this material also affected the values of organic matter removal efficiency for unfiltered effluent samples, which showed abrupt drops along reactor operation, mainly at the last two operation conditions (regions II and III of Fig. 2), where formation of this material was more intense.

Volumetric Loading Rate and Alkalinity Supplementation Optimization

The great variation in bicarbonate alkalinity, observed in region II of Fig. 3, occurred due to the attempt to optimize alkalinity supplementation during reactor operation at volumetric loading rate of 6 g COD l⁻¹ day⁻¹. It can be seen from Figs. 2, 3, and 4 that the system was able to maintain efficiency and stability after reducing alkalinity supplementation from 100 to 50% (day 46 of the assay). During this period, filtered and unfiltered organic matter removal efficiency remained around 90 and 87%, respectively. Volatile acids concentration

Table 4 Average values of the monitored variables in the influent for the three volumetric loading rate conditions^a.

Variable	VLR (g COD l ⁻¹ day ⁻¹)		
	3 ^(a)	6 ^(b)	12 ^(c)
C _{ST} (mg COD l ⁻¹)	988±17 ⁽²³⁾	2,001±14 ⁽⁴¹⁾	4,010±71 ⁽³⁰⁾
TVA (mg HAc l ⁻¹)	47±5 ⁽¹¹⁾	116±7 ⁽¹⁰⁾	204±15 ⁽²²⁾
BA (mg CaCO ₃ l ⁻¹)	182±15 ⁽¹¹⁾	600±17 ⁽¹⁰⁾	1161±55 ⁽²²⁾
pH	8.2±0.2 ⁽¹¹⁾	7.8±0.1 ⁽¹⁰⁾	7.8±0.1 ⁽²²⁾
TS (mg l ⁻¹)	1,324±24 ⁽⁶⁾	2,811±164 ⁽⁴⁾	5,539±10 ⁽¹⁰⁾
TVS (mg l ⁻¹)	1,113±31 ⁽⁶⁾	1,976±81 ⁽⁴⁾	3,728±208 ⁽¹⁰⁾
TSS (mg l ⁻¹)	41±19 ⁽⁶⁾	84±28 ⁽⁴⁾	177±29 ⁽¹⁰⁾
VSS (mg l ⁻¹)	24±10 ⁽⁶⁾	54±17 ⁽⁴⁾	135±26 ⁽¹⁰⁾
SOL (mg COD g TVS ⁻¹ day ⁻¹)	100	180	295

^a Numbers in parentheses refer to the number of samples used for averaging.

(a)/(b)/(c) Duration of the condition (days): 35/66/47

Table 5 Average values of the monitored variables in the effluent for the three volumetric loading rate conditions^a.

Variable	VLR (g COD l ⁻¹ day ⁻¹)		
	3	6	12
C _{ST} (mg COD l ⁻¹)	88±18 ⁽⁷⁾	471±43 ⁽⁹⁾	1,352±87 ⁽¹¹⁾
ε _{ST} (%)	91±2 ⁽⁷⁾	77±2 ⁽⁹⁾	66±2 ⁽¹¹⁾
C _{SF} (mg COD l ⁻¹)	37±15 ⁽⁷⁾	350±36 ⁽⁹⁾	904±50 ⁽¹¹⁾
ε _{SF} (%)	96±2 ⁽⁷⁾	83±2 ⁽⁹⁾	78±1 ⁽¹¹⁾
TVA (mg HAc l ⁻¹)	34±11 ⁽¹¹⁾	219±19 ⁽⁹⁾	512±31 ⁽¹⁵⁾
BA (mg CaCO ₃ l ⁻¹)	215±20 ⁽¹¹⁾	613±58 ⁽⁹⁾	1294±76 ⁽¹⁵⁾
pH	6.5±0.1 ⁽¹¹⁾	6.9±0.1 ⁽¹⁰⁾	7.3±0.1 ⁽¹⁵⁾
TS (mg l ⁻¹)	612±35 ⁽⁶⁾	1,480±74 ⁽³⁾	2,791±76 ⁽¹⁰⁾
TVS (mg l ⁻¹)	404±43 ⁽⁶⁾	654±40 ⁽³⁾	1,086±79 ⁽¹⁰⁾
TSS (mg l ⁻¹)	62±21 ⁽⁶⁾	109±21 ⁽³⁾	217±36 ⁽¹⁰⁾
VSS (mg l ⁻¹)	38±9 ⁽⁶⁾	76±19 ⁽³⁾	175±45 ⁽¹⁰⁾
ROL _F (g COD l ⁻¹ day ⁻¹)	2.85	4.95	9.32
ROL _T (g COD l ⁻¹ day ⁻¹)	2.70	4.59	7.97
V _{CH4} (ml)	137 ⁽¹⁾	189 ⁽¹⁾	304 ⁽¹⁾

^aNumbers in parentheses refer to the number of samples used for averaging.

Biomass present in the reactor (average value for the three conditions): C_{X-TS}=1,582±220 mg TS g foam⁻¹; C_{X-TVS}=1,366±189 mg TVS g foam⁻¹; C_{X-TS}=40±5 g TS l reaction medium⁻¹; C_{X-TVS}=34±5 g TVS l reaction medium⁻¹; C_{X-TVS}/C_{X-TS}=0.86±0.02

in the effluent maintained stability (at around 80 mg HAc l⁻¹) and remained close to those in the influent (Fig. 4).

Considering these results and seeking to optimize the amount of sodium bicarbonate added, alkalinity supplementation was reduced from 50 to 25% on day 50. From thereon, organic matter removal efficiency was seen to drop (Fig. 2) for filtered (from 90 to 86%) and unfiltered effluent samples (from 87 to 84%), concomitant with a progressive increase in total volatile acid concentration, which attained 142.3 mg HAc l⁻¹ on operation day 54, and then 248.39 mg HAc l⁻¹ on day 62, showing onset of system instability. Hence, alkalinity supplementation was increased from 25 to 50% (operation day 65) to avoid loss of system stability and reduce total volatile acid concentration in the effluent.

However, even after increasing alkalinity supplementation to 50%, total volatile acids concentration in the effluent kept increasing over time, reaching 267.1 mg HAc l⁻¹ on day 70 and then 316.2 mg HAc l⁻¹ on day 71. It was then decided, on day 71, to increase alkalinity supplementation in the influent to 100% to eliminate accumulation of total

Fig. 2 Organic matter removal efficiency during reactor operation. I VLR=3 g COD l⁻¹ day⁻¹, II VLR=6 g COD l⁻¹ day⁻¹, III VLR=12 g COD l⁻¹ day⁻¹

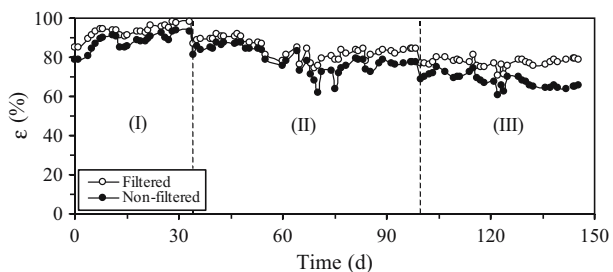
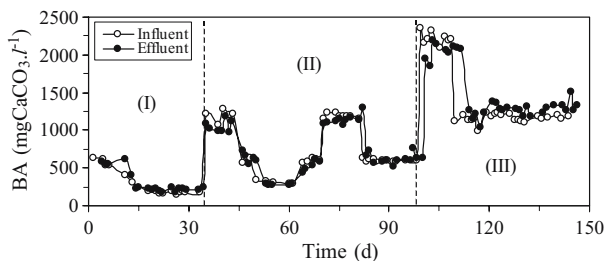


Fig. 3 Bicarbonate alkalinity during reactor operation. *I* VLR=3 g COD $\text{l}^{-1} \text{ day}^{-1}$, *II* VLR=6 g COD $\text{l}^{-1} \text{ day}^{-1}$, *III* VLR=12 g COD $\text{l}^{-1} \text{ day}^{-1}$



volatile acids and to avoid inheriting the instability generated by lack of alkalinity during the unsuccessful attempt to optimize supplementation. Figure 4 shows a drop in total volatile acids concentration in this period, from 312.2 mg HAc l^{-1} , on day 71, to 202.8 mg HAc l^{-1} , on day 82 attaining stability at around 219.0 mg HAc l^{-1} on day 89, where supplementation was again 50%.

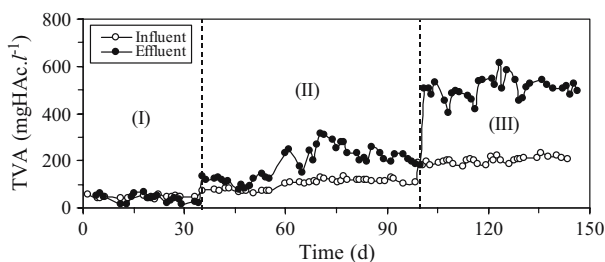
Values of bicarbonate alkalinity in the effluent remained superior to those in the influent for the three conditions, indicating alkalinity production during the entire reactor operation period (Tables 4 and 5). For the first, second, and third operation conditions, alkalinity supplementation to the influent could be optimized to 0.25, 0.50, and 0.50 g NaHCO_3 g COD $^{-1}$, which corresponded to average bicarbonate alkalinity values in the influent of 182, 600, and 1,161 mg CaCO_3 l^{-1} , respectively.

Volumetric Loading Rate and Dynamic Behavior of the Reactor During a Cycle

Figures 5, 6, 7, and 8a illustrate the profiles of the monitored variables at the three volumetric loading rates. Figure 8b illustrates the profile of the main volatile acids identified at the third operation condition (VLR=12 g COD $\text{l}^{-1} \text{ day}^{-1}$). An increase in total volatile acids concentration in the effluent was seen at increasing volumetric loading rate, which remained at around 34, 219, and 512 mg HAc l^{-1} at the first, second, and third operation conditions, respectively (Table 5).

Moreover, contrary to the first operation condition, the last two conditions (VLR=6 and 12 g COD $\text{l}^{-1} \text{ day}^{-1}$) presented average values of total volatile acids concentration in the effluent superior to those in the influent (Tables 4 and 5). This leads to the conclusion that at VLR of 6 and 12 g COD $\text{l}^{-1} \text{ day}^{-1}$ cycle length was insufficient for a higher consumption of volatile acids formed (TVA profiles—Figs. 6 and 7), a fact that can be confirmed by the absence of a plateau in the filtered organic matter concentration profiles for the second and third operation condition (C_{SF} profiles—Figs. 6 and 7). However, despite the increase in total volatile acid concentration in the effluent at higher VLRs, there was no tendency to acids accumulation over time, and pH of the effluent remained close to neutrality, indicating

Fig. 4 Concentration of total volatile acids during reactor operation. *I* VLR=3 g COD $\text{l}^{-1} \text{ day}^{-1}$, *II* VLR=6 g COD $\text{l}^{-1} \text{ day}^{-1}$, *III* VLR=12 g COD $\text{l}^{-1} \text{ day}^{-1}$



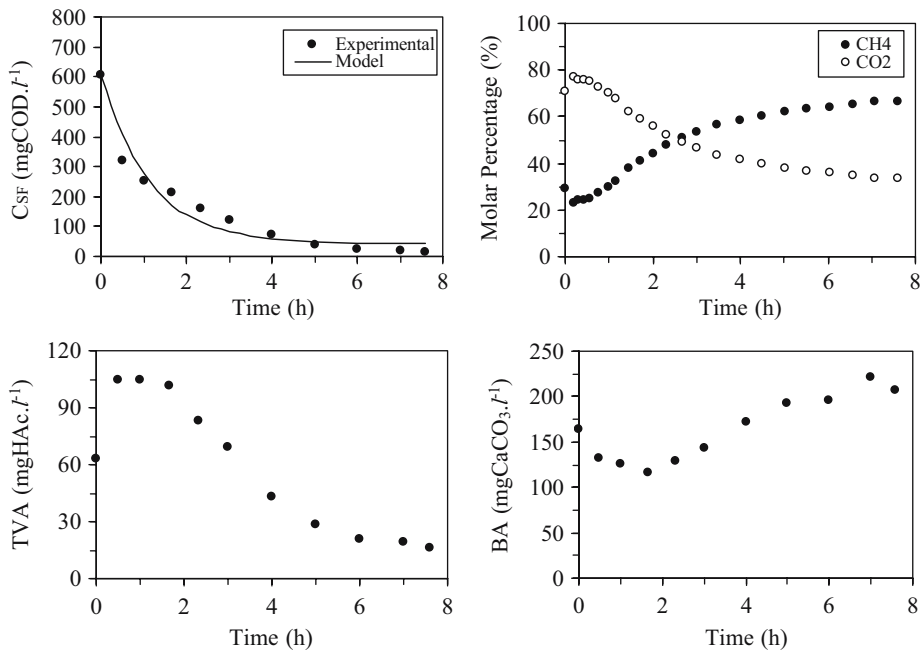


Fig. 5 Time profiles of the main variables for the condition at which $\text{VLR}=3 \text{ g COD l}^{-1} \text{ day}^{-1}$

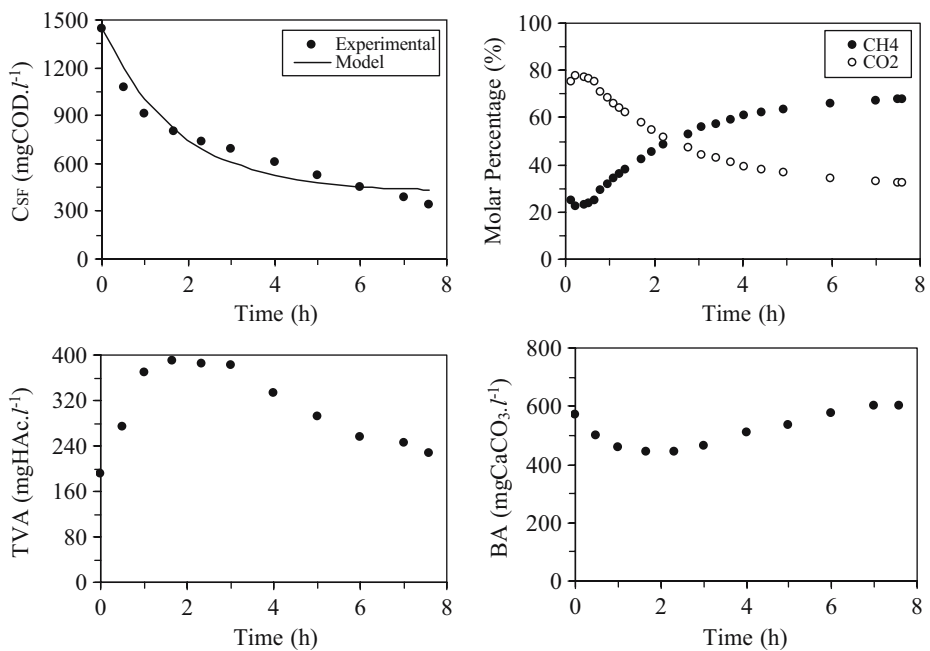


Fig. 6 Time profiles of the main variables for the condition at which $\text{VLR}=6 \text{ g COD l}^{-1} \text{ day}^{-1}$

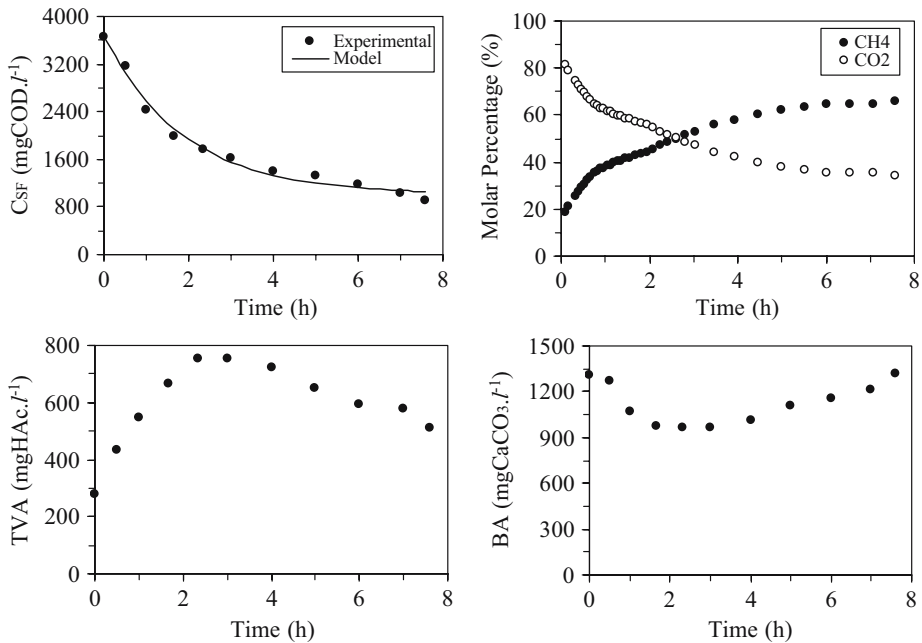


Fig. 7 Time profiles of the main variables for the condition at which $VLR=12 \text{ g COD l}^{-1} \text{ day}^{-1}$

that the alkalinity supplied to the influent was sufficient to neutralize the formed acids and to maintain reactor stability. As to the intermediate volatile acids, the profiles showed that the main acids identified along the cycle were acetic, propionic, and butyric acid (Fig. 8b).

Figure 8a enables verification of methane production along the whole cycle at the three conditions, providing evidence that the reactor was not merely retaining or accumulating organic matter, but rather, degrading the substrate present by anaerobic means. Increasing volumetric loading rate resulted in increase in total methane production (V_{CH_4} —Table 5). This fact might be related to the higher amount of primary substrate supplied to the acidogenic microorganisms at higher VLR, causing increased accumulation of acids along

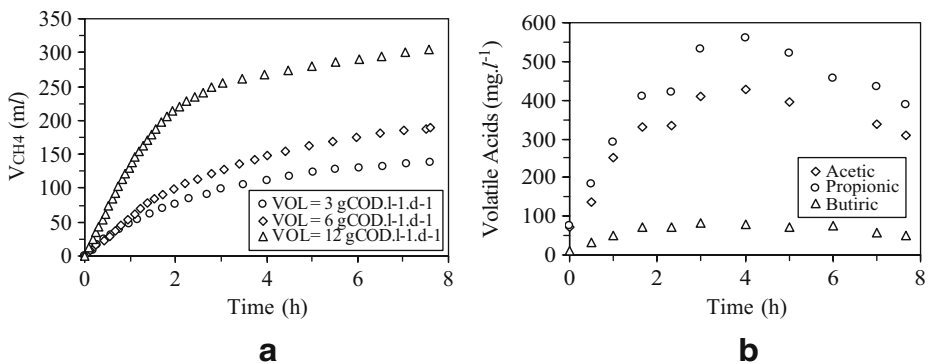


Fig. 8 **a** Time profiles of methane production for the three conditions of VLR and **b** Time profiles of the main volatile acids identified for the condition at which $VLR=12 \text{ g COD l}^{-1} \text{ day}^{-1}$

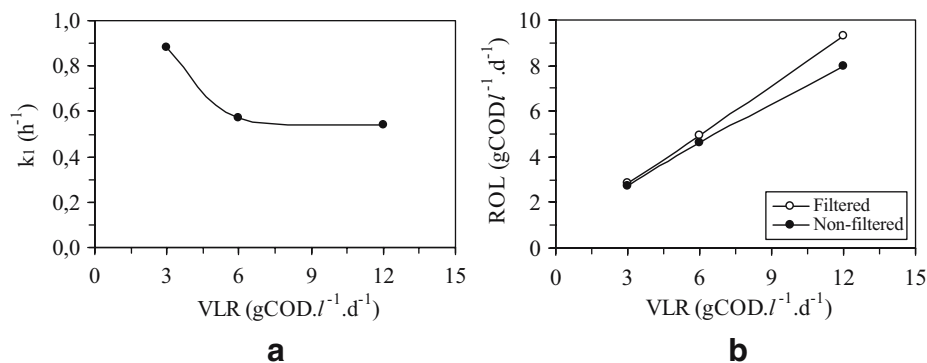


Fig. 9 **a** Apparent first order kinetic parameter and **b** removed organic load as a function of the volumetric loading rate

the cycle and increased supply of acetate to the methanogenic microorganisms, which consequently leads to higher methane production.

This result is confirmed by the increase in removed organic load by the reactor at increasing volumetric loading rate, which amounted to 2.85, 4.95, and 9.32 g COD l^{-1} day $^{-1}$, for filtered effluent samples, and 2.70, 4.59, and 7.97 g COD l^{-1} day $^{-1}$, for unfiltered effluent samples, at VLRs of 3, 6, and 12 g COD l^{-1} day $^{-1}$, respectively (Table 5 and Fig. 9b). That is, as volumetric loading rate is increased, the higher will be the amount of organic matter removed from the wastewater and converted to methane. However, it should be pointed out that although in quantitative terms, the amount of organic matter removed per cycle has been higher at higher VLR, in qualitative terms, the increase in VLR caused a drop in effluent quality (C_{SF} and C_{ST} —Table 5), i.e., an increase in organic matter concentration in the effluent, which was around 37, 350, and 904 mg COD l^{-1} , for filtered effluent samples, and 88, 471, and 1,352 mg COD l^{-1} , for unfiltered effluent samples, at VLRs of 3, 6, and 12 g COD l^{-1} day $^{-1}$, respectively.

Volumetric Loading Rate and Kinetic Model Fitting

Table 6 and Fig. 9a show that the first operation condition (VLR=3 g COD l^{-1} day $^{-1}$) presented the highest apparent kinetic constant, which was equal to 0.88 h^{-1} . At this condition, the organic matter removal efficiency was 91 and 96%. Moreover, the time required for the filtered organic matter concentration in the reactor to stabilize was 6 h. Increasing VLR to 6 g COD l^{-1} day $^{-1}$ at the second condition and to 12 g COD l^{-1} day $^{-1}$, at the third condition, resulted in a drop in the apparent kinetic constant, which remained around 0.57 h^{-1} for both conditions. In the same way, it is worth to mention that the organic

Table 6 Values obtained from fitting the kinetic model to the experimental organic matter concentration profile data.

VLR (g COD l^{-1} day $^{-1}$)	C_{SIO} (mg COD l^{-1})	k_1 (h^{-1})	C_{SR} (mg COD l^{-1})	r^2
3	607	0.88±0.12	41	0.954
6	1,448	0.57±0.08	421	0.954
12	3,646	0.54±0.05	1,019	0.986

matter removal efficiency also decreased to 77 and 83% ($\text{VLR}=6 \text{ g COD l}^{-1} \text{ day}^{-1}$) and 66 and 78% ($\text{VLR}=12 \text{ g COD l}^{-1} \text{ day}^{-1}$), for nonfiltered and filtered samples, respectively.

The reduction in the apparent kinetic constant might be related to the increase in maximum concentration of total volatile acids (peak of total volatile acids) along the cycle at increasing VLR (TVA profiles—Figs. 5, 6, and 7). For the second and third operation conditions ($\text{VLR}=6$ and $12 \text{ g COD l}^{-1} \text{ day}^{-1}$), the maximum concentration of total volatile acids reached levels of $391 \text{ mg HAc l}^{-1}$ and $754 \text{ mg HAc l}^{-1}$, respectively, which might have caused some type of inhibition to the biomass, resulting in reduction in the kinetic constant value.

Volumetric Loading Rate Effects of Shock Load

At all conditions, the profiles taken in the cycle subsequent to the shock loads presented, in general, the same tendency and behavior of those taken before the shock. This fact can be corroborated by the organic matter removal efficiencies, in the cycles subsequent to the shock load, of 98, 85, and 79%, for filtered effluent samples, and of 93, 78, and 65%, for unfiltered effluent samples, for the first, second and third operation conditions, respectively. The profiles taken in the cycle after the shock load also showed maintenance of the values of total volatile acids concentration and bicarbonate alkalinity in the effluent, at approximately 22, 195, and $478 \text{ mg HAc l}^{-1}$, and 246, 762, and $1,514 \text{ mg CaCO}_3 \text{ l}^{-1}$, at the first, second, and third conditions, respectively.

Microbiology Analysis

Total volatile solid concentration per gram of foam ($C_{\text{X-TVVS}}$) remained between 1,148 and $1,482 \text{ mg TVS g foam}^{-1}$ at the three conditions, i.e., no biomass loss by the support occurred during the proposed operation conditions. Total mass of biomass in the reactor, in the form of total volatile solids, remained at around 80 g TVS . Ratios of $C_{\text{X-TVVS}}/C_{\text{X-TS}}$ varied little: between 0.85 and 0.88. Microbiological analyses showed the existence of bacilli-like and vibria-like cells inside the support, as well as morphologies similar to *Methanosarcina* sp. and *Methanosaeta* sp., with the last two showing to be in equilibrium.

Conclusions

Despite alkalinity deficiency and high biodegradability of cheese whey, the system responded well to the increase in volumetric loading rate and to decrease in alkalinity supplementation, maintaining stability at all conditions. Increase in volumetric loading rate for long periods resulted in a drop in organic matter removal efficiency and increase in total volatile acid concentration of the effluent. Alkalinity supplementation to the influent could be reduced to 75, 50, and 50% at volumetric loading rate of 3, 6, and $12 \text{ g COD l}^{-1} \text{ day}^{-1}$, respectively, showing it to be sufficient to guarantee process stability. Application of one-cycle shock loads of 6, 12, and $24 \text{ g COD l}^{-1} \text{ day}^{-1}$ did not cause drop in reactor efficiency and stability. Addition of salts to the influent improved reactor efficiency. Formation of a viscous polymer-like material resulted in a drop in organic matter removal efficiency for unfiltered effluent samples.

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