

Effect of Organic Load on the Performance and Methane Production of an AnSBBR Treating Effluent from Biodiesel Production

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Abstract Currently, there is an increasing demand for the production of biodiesel and, consequently, there will be an increasing need to treat wastewaters resulting from the production process of this biofuel. The main objective of this work was, therefore, to investigate the effect of applied volumetric organic load (AVOL) on the efficiency, stability, and methane production of an anaerobic sequencing batch biofilm reactor applied to the treatment of effluent from biodiesel production. As inert support, polyurethane foam cubes were used in the reactor and mixing was accomplished by recirculating the liquid phase. Increase in AVOL resulted in a drop in organic matter removal efficiency and increase in total volatile acids in the effluent. AVOLs of 1.5, 3.0, 4.5 and 6.0 gCOD L⁻¹ day⁻¹ resulted in removal efficiencies of 92%, 81%, 67%, and 50%, for effluent filtered samples, and 91%, 80%, 63%, and 47%, for non-filtered samples, respectively, whereas total volatile acids concentrations in the effluent amounted to 42, 145, 386 and 729 mg HAC L⁻¹, respectively. Moreover, on increasing AVOL from 1.5 to 4.5 gCOD L⁻¹ day⁻¹ methane production increased from 29.5 to 55.5 NmL CH₄g COD⁻¹. However, this production dropped to 36.0 NmL CH₄g COD⁻¹ when AVOL was increased to 6.0 gCOD L⁻¹ day⁻¹, likely due to the higher concentration of volatile acids in the reactor. Despite the higher concentration of volatile acids at the highest AVOL, alkalinity supplementation to the influent, in the form of sodium bicarbonate, at a ratio of 0.5–1.3 gNaHCO₃g COD_{fed}⁻¹, was sufficient to maintain the pH near neutral and guarantee process stability during reactor operation.

Keywords AnSBBR · Applied volumetric organic load · Methane production · Biodiesel effluent

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Introduction

The anaerobic sequencing batch biofilm reactor (AnSBBR), i.e., containing immobilized biomass, is an important option of the anaerobic treatment of wastewaters. Utilization of inert supports allows improved biomass retention, elimination of the sedimentation step, and reduces total cycle length. However, the occurrence of dead zones, short circuits, and mass transfer resistance due to organic overload are some of the problems that affect treatment performance. Investigations on the application of this bioreactor configuration to the treatment of different effluents contribute to improved understanding of the process. According to Zaiat et al. [41], investigations on anaerobic sequencing batch reactor (ASBR) and AnSBBR configurations should include the influence of fundamental and technological aspects that usually affect reactor efficiency and stability. Fundamental aspects include: mixing gradient, ratio between substrate and biomass concentration (F/M), geometric configuration of the reactor and feed strategy. Technological aspects include: application to domestic and industrial wastewaters with different characteristics in terms of composition.

Several studies on the influence of different process variables have been encountered in the literature with main objective the qualitative and quantitative knowledge of the relation of these variables to efficiency and stability and, consequently, system optimization. Investigation on the effect of agitation, which is related to mass transfer resistance of the liquid phase to the biomass, has been dealt with in some works, accomplished either by recirculation of the liquid phase [6, 7, 25] or by mechanical stirring [1, 12, 17, 27, 28]. Fill time or feed strategy is another important variable that has been investigated and which may affect process performance by the availability of the substrate in the wastewater, by inhibition or toxicity of effluents containing high organic load or lacking nutrients and/or alkalinity [2, 4, 23].

The aforementioned works emphasize the importance of fundamental studies on the behavior of ASBRs and AnSBBRs provided with mechanical stirring or recirculation of the liquid phase. Another line of investigation deals with technological studies on these bioreactors of which the main objective is the analysis of process efficiency and stability when increasing organic loads applied to different wastewaters such as synthetic wastewater [8–10, 18], piggery wastewater [1, 19], cheese whey [3, 11], landfill leachate [16], effluent from automobile industry [21], effluents from food and beverage plants [5, 37], effluents from personal care industry [22] and effluents from chemical industries [30, 32, 33].

Currently, a potential need to treat wastewaters resulting from the production processes of biofuels has been observed in the literature. The anaerobic biological treatments should be considered, especially because in addition to removing organic matter they produce methane from the generated biogas, rendering the biofuel production process economically more advantageous [39]. Some works have appeared in the literature related to the anaerobic treatment of effluent from the production process of biodiesel [14, 20, 29, 35, 38].

Within this context, the main objective of this work was to investigate the effect of AVOL, by varying the organic matter concentration in the influent of the reactor, on the efficiency and stability of an anaerobic sequencing batch biofilm reactor containing polyurethane foam cubes and mixed by recirculating the liquid phase, applied to the treatment of effluent from biodiesel production, aiming at organic matter removal as well as methane production.

Materials and Methods

Experimental Set-up

The reactor, shown in Fig. 1, consisted of an acrylic cylindrical column with total capacity of 3.7 L, 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 of: (1) a side reservoir with total capacity of 2.0 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. Feeding and discharge were performed using diaphragm pumps equipped with automatic timers. The chamber in which the reactor remained was kept at

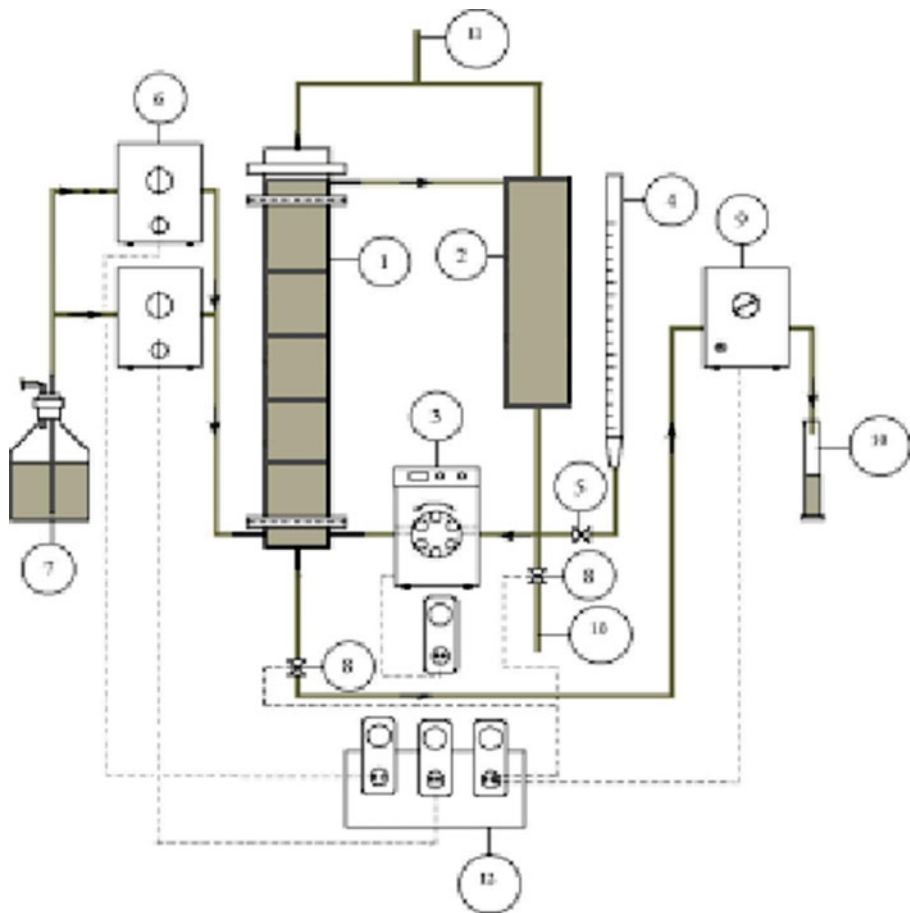


Fig. 1 Schematic representation of the system used for treating biodiesel production effluent (1 reactor containing immobilized biomass, 2 circulation reservoir, 3 circulation pump, 4 flow rate meter, 5 valve, 6 feed pumps, 7 wastewater reservoir, 8 discharge valve, 9 discharge pump, 10 effluent outlet, 11 biogas outlet, 12 control unit; straight lines represent hydraulic lines, dashed lines represent power lines)

$30 \pm 1^\circ\text{C}$ by a heating system composed of resistances and fans, as well as a temperature sensor and controller.

The inert support containing the immobilized biomass was placed between perforated stainless steel plates, which divided the 540-mm high reactor in five equal parts (108 mm) to avoid bed compacting. A 20-mm compartment at the bottom of the reactor (0.13 L—external diameter of 100 mm and wall thickness of 3.5 mm) allowed enhanced distribution of the wastewater, preventing formation of preferential routes. At the upper part of the reactor, a 40-mm region (0.27 L—external diameter of 100 mm and wall thickness of 3.5 mm) functioned as a biogas collector (CH_4 and CO_2).

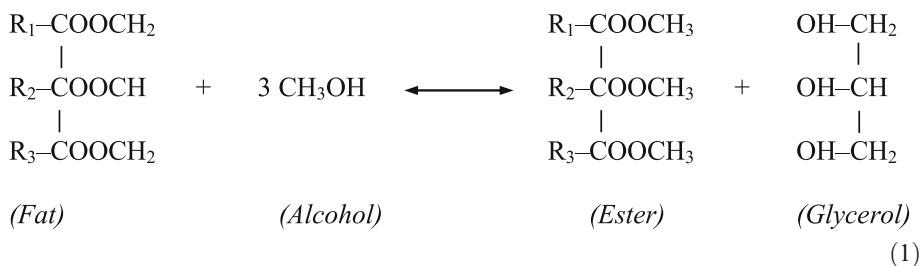
Inoculum and Inert Support

The inoculum used in all experiments came from an up-flow anaerobic sludge blanket reactor treating wastewater from a poultry slaughterhouse. Immobilization procedure, after Zaiat et al. [40], 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 and total solids concentration of 51 and 62 g L^{-1} , respectively.

The inert support used consisted of 1-cm polyurethane foam cubes with apparent density of 23 kg m^{-3} and porosity of 95%. One of the main advantages of using polyurethane foam is that its high porosity allows immobilization of a significant amount of biomass that does not become detached during the charge, discharge, and reaction steps while the liquid phase is recirculated [36].

Wastewater

The wastewater used was prepared by diluting effluent from the biodiesel production process considering only the production stage. That is, the effluent was obtained by separating the formed biodiesel from the byproducts in the reaction stage and consisted primarily of glycerin as well as non-reacted soy oil and methanol. Biodiesel was produced on laboratory scale from soy oil and methanol. In a simplified manner, biodiesel is produced from the transesterification of fats, according to the following scheme.



In soybean oil radicals R_1 , R_2 , and R_3 have an average molecular weight of 880 g mol^{-1} [15]. Methanol, biodiesel (ester), and glycerol (glycerin) have molecular weights of 32, 884, and 92 g mol^{-1} respectively. Densities of soybean oil and methanol at the conditions of this work ($20\text{--}60^\circ\text{C}$ and $0.9\text{--}1.0 \text{ atm}$) were assumed to be 0.92 and 0.79 kg L^{-1} , respectively [24].

The stoichiometric ratio required for 100% conversion of the reagents is 1:3 between the fat (soybean oil) and the alcohol (methanol). However, to shift the chemical equilibrium towards biodiesel formation, a 1 to 5 ratio was used. Assuming complete conversion of available soybean oil for the reaction and considering the amount produced biodiesel as basis for calculation, the ratio of byproducts was 10.4% ($92 \div 884$) in relation to generated glycerin and 7.2% ($2 \times 32/884$) in relation to unreacted methanol, i.e., approximately 18% of byproducts are generated in relation to the mass of produced biodiesel. Within this context, these byproducts made up the major part of the wastewater used, which was prepared by dissolving the formed glycerin in tap water.

The theoretical COD of the glycerin ($C_3H_8O_3$ —92 g mol^{-1}) is approximately 1.2 g COD g glycerin^{-1} ($C_3H_8O_3 + 7/2 O_2 \rightarrow 3CO_2 + 4H_2O$), but the experimental ratio measured was approximately 0.6–0.8 g glycerin which corresponded to 1 g COD. The difference occurred due to non-reacted fat in the reaction performed in laboratory scale to produce the residual used in the assays.

Analytical Methods and Microbiological Exams

The operating variables monitored according to *Standard Methods for Examination of Water and Wastewater* [34] were as follows: organic matter concentration (measured as COD) for non-filtered (C_{ST}) and filtered samples (C_{SF}), bicarbonate alkalinity (BA), total volatile acids concentration (C_{TVA}), total solids concentration (C_{TS}), total volatile solids concentration (C_{TVS}), total suspended solids concentration (C_{TSS}), and volatile suspended solids concentration (C_{VSS}), considering also the method proposed by Dilallo and Albertson [13], which was modified by Ripley et al. [26] to determine alkalinity.

The intermediate volatile acids concentrations (C_{IVA}) were analyzed using a Hewlett Packard® 6890 gas chromatograph equipped with a flame ionization detector and Hewlett Packard® Innowax column of 30 m \times 0.25 mm \times 0.25 μm . Hydrogen was used as drag gas at a flow rate of 2.0 mL min^{-1} . Injector temperature was 250°C, split ratio 20 and injection volume was 1.0 μL . The oven was set at 100°C for 3 min with a heating ramp of 5°C min^{-1} up to 180°C and held there for 5 min. A post run followed of 200°C for 3 min. Detector temperature was 300°C. Synthetic air, hydrogen, and nitrogen make-up flow rate were 300, 30, and 35 mL min^{-1} , respectively.

Samples of biogas generated by anaerobic degradation were collected in two ways (both at a 40-mm region in the upper part of the reactor which functioned as a biogas collector):

- Biogas concentration (concentration of methane and carbonic gas in the biogas— C_{CH_4} and C_{CO_2}) was measured using a syringe (chromatography syringe type for gas analysis) and a Hewlett Packard® 6890 gas chromatograph equipped with a thermal conductivity detector, in which the sample volume was 1 mL, 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;
- Biogas production (volumetric methane production— V_{CH_4}) was measured using a flexible silicone tube connected to a closed Erlenmeyer containing NaOH (50 g L^{-1} to promote CO_2 absorption) and recording the displacement of a graduated tube connected to the Erlenmeyer. To avoid large displacements, the system was purged after each sampling. The accumulated volume of biogas produced was corrected to “STP” (273 K and 1 atm).

At the end of each operation condition, bioparticle samples were withdrawn from the reactor for two purposes:

- For microbiological exams, which were carried out by means of common optical and fluorescence phase contrast microscopy, using a BX41 Olympus® microscope;
- For analysis of solids relative to the immobilized biomass in the reactor, i.e., analysis of concentrations of total solids (S_{TS}) and total volatile solids (S_{TVS}) in the reactor, enabling in this way to estimate the biomass concentration in the reactor as S_{TVS} as follows: polyurethane foam (five cubes) was collected from the reactor, washed with distilled water and wash water collected in porcelain capsules after which it was possible to calculate values of total solids and total volatile solids (according to [34]) per gram of foam contained in the reactor (the washed foam cubes were then dried at 105°C for 24 h to determine the dry weight), obtaining the amount and concentration of biomass in the reactor.

Operating Variables

For each operating condition studied, the AVOL was calculated for non-filtered influent samples and the removed volumetric organic load (RVOL) was calculated for filtered and non-filtered effluent samples. AVOL and RVOL can be defined as the amount of organic matter applied and removed, respectively, by the reactor per time unit and per reactor medium volume, for example, g COD L⁻¹ day⁻¹. For batch reactors applied AVOL, removed volumetric organic load for filtered (RVOL_{SF}) and non-filtered (RVOL_{ST}) effluent samples was calculated using Eqs. 2, 3, and 4 respectively, where C_{SINF} is the organic matter concentration in the influent, C_{SF} is the filtered organic matter concentration in the effluent, C_{ST} is the non-filtered organic matter concentration in the effluent, V_A is the volume of wastewater fed per cycle, V is the reaction medium volume of the system, and n the number of cycles per day.

$$AVOL = \frac{C_{SINF} \cdot n \cdot V_A}{V} \quad (2)$$

$$RVOL_{SF} = \frac{(C_{SINF} - C_{SF}) \cdot n \cdot V_A}{V} \quad (3)$$

$$RVOL_{ST} = \frac{(C_{SINF} - C_{ST}) \cdot n \cdot V_A}{V} \quad (4)$$

The applied (ASOL) and removed (RSOL) specific organic load may be defined as the amount of organic matter applied and removed, respectively, by the reactor per time unit and amount of biomass in the reactor (mg COD gTVS day⁻¹). For batch reactors, the applied and removed specific organic load for filtered (RSOL_{SF}) and non-filtered (RSOL_{ST}) effluent samples were calculated using Eqs. 5, 6, and 7, respectively, where M_{TVS} is the total volatile solids mass relative to the immobilized biomass in the reactor that estimates the biomass concentration in the reactor.

$$\overline{\text{ASOL}} = \frac{C_{\text{SINF}} \cdot n \cdot V_A}{M_{\text{TVS}}} \quad (5)$$

$$\text{RSOL}_{\text{SF}} = \frac{(C_{\text{SINF}} - C_{\text{SF}}) \cdot n \cdot V_A}{M_{\text{TVS}}} \quad (6)$$

$$\text{RSOL}_{\text{ST}} = \frac{(C_{\text{SINF}} - C_{\text{ST}}) \cdot n \cdot V_A}{M_{\text{TVS}}} \quad (7)$$

The relation between volumetric production of methane and the mass of removed organic matter (i.e., consumed organic matter) per cycle was also calculated at each operation condition for filtered effluent samples ($N \text{ mL CH}_4\text{g COD}^{-1}$) at standard temperature and pressure (STP: 0°C and 1 atm), according to Eq. 8:

$$Y_{\frac{\text{CH}_4}{\text{CDO}}} = \frac{V_{\text{CH}_4}}{(C_{\text{SINF}} - C_{\text{SF}}) \cdot V_A} \quad (8)$$

Kinetic Model and Mathematical Fit

The kinetic model used in this work was developed by Rodrigues et al. [27] based on the model of Bagley and Brodtkorb [2]. The latter is an adaptation of another model considered by the International Water Association and applied to treatment systems with activated sludge. In the adopted kinetic model, developed for the anaerobic sequencing batch biofilm reactor, the anaerobic process of organic matter degradation was simplified in two consecutive steps. In the first one the substrate (S) is converted to total volatile acids (TVA), and in the second these same acids are transformed into methane (M). In the two stages, the conversion reactions were considered as being first-order. The model also admits the existence of a residual substrate concentration and total volatile acids in which the respective reaction rates were zero. Equations 9 to 12 present the organic matter degradation route and the reaction rate equations of substrate (r_S) consumption, formation and consumption of total volatile acids (r_{TVA}), and methane formation (r_M) respectively.



$$r_S = k_{1S} \cdot (C_S - C_{\text{SR}}) \quad (10)$$

$$r_{\text{TVA}} = k_{1\text{TVA}} \cdot (C_S - C_{\text{SR}}) - k_{2\text{TVA}} \cdot (C_{\text{TVA}} - C_{\text{TVAR}}) \quad (11)$$

$$r_M = k_{2M} \cdot (C_{\text{TVA}} - C_{\text{TVAR}}) \quad (12)$$

In Eq. 9, letters a , b , and c stand for stoichiometric coefficients, and k_1 and k_2 stand for the apparent kinetic parameters of the adopted model. In Eqs. 10, 11, and 12 k_{1S} , $k_{1\text{TVA}}$, $k_{2\text{TVA}}$, and k_{2M} are the same apparent kinetic parameters associated with the substrate consumption, total volatile acids formation and consumption, and methane formation

respectively. C_S and C_{TVA} are the substrate concentration and total volatile acids concentration, and C_{SR} and C_{TVAR} the residual values of these matters where the reaction rate is zero.

The kinetic parameter “ k ” is related to the reaction rate, indicating a relation to the time necessary for the concentration (S, TVA, or M) to reach a residual value in accordance with the hypothesis of the kinetic model.

Subscripts “1 or 2” are related to the reaction (1: COD \rightarrow TVA/2: TVA \rightarrow M).

Subscripts “S, TVA or M” are related to the experimental values used to calculate the parameters (k_{1S} : calculated from the values S/k_{1TVA} and k_{2TVA} : calculated from the values TVA/k_{2M} : calculated from the values M).

Equations 13 to 15 present the mass balance of the reactor in batch mode concerning the kinetic model (substrate, total volatile acids and methane), where C_S , C_{TVA} , and C_M correspond to the concentrations of substrate, total volatile acids and methane in the liquid phase, respectively, and C_{S0} and C_{TVA0} the respective feed values and V the reaction volume. These equations were used to determine the kinetic parameters k_{1S} , k_{1TVA} , k_{2TVA} , k_{2M} , C_{SR} , and C_{TVAR} of the model. To deal with the differential equations, the Euler numerical integration method (Excel® software) was used. These parameters were calculated using as objective function in the optimization procedure (function *Solver* of the Excel® software) the minimum square error between experimental and model data.

$$\frac{dC_S}{dt} = -r_S \quad (13)$$

$$\frac{dC_{TVA}}{dt} = +r_{TVA} \quad (14)$$

$$\frac{dC_M}{dt} = +r_M \quad (15)$$

It should be mentioned that the methane concentration in the liquid (C_M —mmol $\text{CH}_4 \text{ L}^{-1}$) was not measured but calculated through the molar methane production (mmol CH_4), calculated by the volumetric methane production (mL CH_4) and the liquid medium in the reactor (L). Thus, C_M is a ratio between molar methane production and liquid medium in the reactor, which is done to turn this into an intensive variable, i.e., proportional to the volume treated per cycle.

Experimental Protocol

The study regarding the influence of applied volumetric organic load on reactor stability and efficiency was carried out by increasing the organic matter concentration in the influent. Four influent concentrations have been studied: 1.0, 2.0, 3.0, and 4.0 gCOD L^{-1} , which corresponded to AVOLs of 1.5, 3.0, 4.5, and 6.0 $\text{gCOD L}^{-1} \text{ day}^{-1}$, respectively. The reactor was operated at approximately $30 \pm 1^\circ\text{C}$ in 8-h cycles, i.e., three cycles a day. The total volume (V) of reaction medium in the system was 3.0 L: 1.5 L in the reactor and 1.5 L in the side reservoir. At the beginning of an operation cycle, the side reservoir was fed with a volume (V_A) of 1.5 L within 10 min. Next, recirculation of the medium was started at a velocity of 0.19 cm s^{-1} for 460 min. The value of this velocity was defined in a previous work [25]. After this period, at the end of the cycle, recirculation was interrupted and 1.5 L

medium was discharged into the side reservoir in 10 min. Hence, the fed/discharged volume in each cycle or renewed volume corresponded to the volume of medium in the side reservoir, that is, 1.5 L (50% of the volume of total reaction medium). The remaining medium, denominated residual volume, corresponding to 1.5 L (50% of the volume of total reaction medium) was maintained in the reactor. After reactor discharge, a time interval of 1 min was allowed as safety period for synchronizing the operation of the timer-controlled feed and discharge pumps, and then the next cycle was started.

At this point, it is important to mention that the volume of the reaction medium inside the reactor was 1.5 L (total volume 3.7 L) due to the foam containing the biomass and the perforated stainless steel plates). Due to safety reasons (as overflow) the volume of the reaction medium inside the reservoir was 1.5 L (total volume 2.0 L). Furthermore, utilization of inert support makes clarification unnecessary.

Supplementation of alkalinity was carried out as follows: at startup of each investigated condition, alkalinity was supplemented to the influent at a ratio $\text{g NaHCO}_3/\text{g COD}_{\text{fed}}$ of 0.50. When the system presented signs of instability, such as increase in concentration of organic matter and total volatile acids in the effluent, this relation $\text{g NaHCO}_3/\text{g COD}_{\text{fed}}$ was increased in the influent to enable stabilization of the values of these variables in the effluent and hence system stability. The system presented “signs of instability” when significant increase was observed in concentration of organic matter and total volatile acids in the effluent, considering the behavior of these monitored process variables during the experimental period.

After reaching stability, verified by the attainment of approximately constant values of the monitored effluent parameters at final cycle condition, profiles were run of the following: filtered organic matter concentration (C_{SF}), bicarbonate alkalinity (BA), total volatile acids concentration (C_{TVA}), intermediate volatile acids concentration (C_{IVA}), volumetric methane production (V_{CH_4}), methane concentration in the liquid (C_{M}) and the gas phase (C_{CH_4}), carbonic gas concentration in the gas phase (C_{CO_2}), and their molar percentages in the gas phase ($\%\text{CH}_4$ – $\%\text{CO}_2$). These profiles allowed better understanding of the organic matter degradation routes during an AnSBBR operation cycle, as well as estimation of kinetic parameters by fitting the kinetic model to these profiles. Samples were taken at different time intervals during a cycle, taking care to not exceed 10% of the total volume of wastewater in the system, i.e., collected volume was always less than 300 mL.

Results and Discussion

Tables 1 and 2 show the average values of the monitored variables in the influent and effluent of the reactor at the four investigated conditions, respectively. Increase in AVOL resulted in increase in effluent concentration of the reactor and a drop in organic matter removal efficiency. Efficiencies were 92%, 81%, 67%, and 50% for filtered effluent samples and 91%, 80%, 63%, and 47% for non-filtered effluent samples at AVOL values of 1.5, 3.0, 4.5, and 6.0 $\text{gCOD L}^{-1} \text{ day}^{-1}$, respectively. Total volatile acids concentration in the effluent increased simultaneously and remained around 42, 145, 386, and 729 mg HAc L^{-1} , respectively. The difference between applied (AVOL) and removed volumetric (RVOL) organic load is defined by the organic removal efficiency (ϵ) for both non-filtered samples and filtered samples. Thus, when the organic removal efficiency is close to non-filtered and filtered samples then the respective removed volumetric load would be too. Furthermore, the low standard deviation (in organic removal efficiency) permitted to conclude that there is no significant difference for all assays.

Table 1 Average values of the monitored variables in the influent for the four applied volumetric organic load operational conditions

Variable	AVOL (g COD L ⁻¹ day ⁻¹)			
	1.5 (43 days)	3.0 (40 days)	4.5 (38 days)	6.0 (39 days)
C _{ST} (mg COD L ⁻¹)	980±21 (11)	2,065±76 (10)	3,065±77 (23)	3,954±312 (4)
C _{TVA} (mg HAc L ⁻¹)	36±4 (11)	48±2 (5)	107±8 (14)	58±6 (10)
BA (mg CaCO ₃ L ⁻¹)	472±13 (11)	907±12 (5)	2,802±101 (14)	3,759±414 (10)
pH	8.5±0.1 (11)	8.5±0.1 (5)	8.5±0.1 (14)	8.6±0.1 (10)
C _{TS} (mg TS L ⁻¹)	1,097±108 (4)	2,059±246 (4)	4,228±411 (5)	4,711±1,070 (4)
C _{TVS} (mg TVS L ⁻¹)	618±80 (4)	1,059±178 (4)	1,645±225 (5)	1,878±148 (4)
C _{TSS} (mg TSS L ⁻¹)	37±14 (4)	41±9 (4)	58±19 (5)	226±87 (4)
C _{VSS} (mg VSS L ⁻¹)	26±16 (4)	30±4 (4)	41±18 (5)	169±72 (4)
V (L)	1.5±0.2 (11)	1.5±0.2 (11)	1.5±0.2 (11)	1.5±0.2 (11)
gNaHCO ₃ g COD _{fed} ⁻¹	0.50	0.50	1.30	1.30

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

The solids removal efficiency obtained in the assays presented standard deviation values that did not permit security performance comparison between the different AVOLs implemented.

The increase in organic matter concentration, with consequent drop in removal efficiency and increase in total volatile acids in the effluent as a result of increasing AVOL, can be clearly seen from Figs. 2, 3, and 4, respectively. Figures 5, 6, 7, and 8 illustrate the profiles of the main monitored variables (experimental points and curves of the fitted kinetic model), whereas Fig. 9a and b illustrate, respectively, the values of volumetric methane production and the relationship between volumetric methane production and mass of removed organic matter per cycle as a function of the AVOL.

At AVOL of 1.5 and 3.0 gCOD L⁻¹ day⁻¹, alkalinity supplementation to the influent was 0.50 gNaHCO₃g COD_{fed}⁻¹, which corresponded to average bicarbonate alkalinity in the influent of 472 and 907 mg CaCO₃L⁻¹, respectively, whereas at AVOL of 4.5 and 6.0 g COD L⁻¹ day⁻¹ alkalinity supplementation to the influent was 1.30 gNaHCO₃·gCOD_{fed}⁻¹, which corresponded to average bicarbonate alkalinity in the influent of 2,802 and 3,759 mg CaCO₃L⁻¹, respectively.

Total solids (S_{TS}) and total volatile solids concentration (S_{TVS}) per liter reaction medium remained around 61 gTS L⁻¹ and 50 gTVS L⁻¹, respectively, during the entire operation period. Total solids (S'_{TS}) and total volatile solids concentration (S'_{TVS}) per gram foam were 1,543 mg TS g foam⁻¹ and 1,261 mg TVS g foam⁻¹, respectively, at the four conditions, i.e., no biomass loss occurred at these proposed conditions. Microbiological analyses showed the existence of bacilli- and vibria-like cells inside the support, as well as morphologies similar to *Methanosarcina* sp. and *Methanosaeta* sp., with the last two in equilibrium.

Under all conditions, average total volatile acids concentrations in the effluent were superior to those in the influent. This difference increased considerably as AVOL increased (Fig. 4). Hence, at the second, and mainly at the last two conditions of higher AVOL, cycle length was too short for improved consumption of the formed TVA (TVA profiles—Figs. 6, 7, and 8). This can be clearly seen by the absence of a plateau in the organic matter concentration profiles at the last three operation conditions (C_{SF} profiles—Figs. 6, 7, and 8). However, despite the increase in TVA concentration in the effluent as a function of increasing AVOL there was no accumulation of acids at the four proposed conditions and effluent pH was in the range of 7.3 to 8.2 during the 140-day assay, indicating that the bicarbonate alkalinity supplied to the influent was sufficient to neutralize the formed acids,

Table 2 Average values of the monitored variables in the effluent for the four applied volumetric organic load operational conditions

Variable	AVOL (g COD L ⁻¹ day ⁻¹)			
	1.5	3.0	4.5	6.0
C_{ST} (mg COD L ⁻¹)	89±12 (7)	411±11 (10)	1,142±100 (13)	2,084±73 (7)
ε_{ST} (%)	91±1 (7)	80±0 (10)	63±3 (13)	47±2 (7)
C_{SF} (mg COD L ⁻¹)	76±11 (7)	400±10 (10)	1,011±89 (13)	1,956±60 (7)
ε_{SF} (%)	92±1 (7)	81±0 (10)	67±3 (13)	50±2 (7)
C_{TVA} (mg HAc L ⁻¹)	42±12 (9)	145±10 (8)	386±33 (13)	729±32 (9)
BA (mg CaCO ₃ L ⁻¹)	372±19 (9)	758±25 (8)	2,475±50 (13)	3,204±81 (9)
pH	7.3±0.1 (11)	7.6±0.2 (8)	7.9±0.2 (13)	8.2±0.2 (9)
C_{TS} (mg TS L ⁻¹)	994±162 (4)	1,843±125 (4)	4,035±443 (6)	5,237±417 (4)
C_{TVS} (mg TVS L ⁻¹)	545±93 (4)	872±83 (4)	1,555±262 (6)	1,673±112 (4)
C_{TSS} (mg TSS L ⁻¹)	25±12 (4)	37±10 (4)	38±8 (6)	74±10 (4)
C_{VSS} (mg VSS L ⁻¹)	13±6 (4)	24±4 (4)	32±17 (6)	34±5 (4)
ASOL (mg COD gTVS ⁻¹ day ⁻¹)	29.9 (7)	60.1 (10)	90.0 (13)	119.8 (7)
RVOL _{SF} (g COD L ⁻¹ day ⁻¹)	1.36 (7)	2.49 (10)	3.08 (13)	3.00 (7)
RVOL _{ST} (g COD L ⁻¹ day ⁻¹)	1.34 (7)	2.48 (10)	2.88 (13)	2.81 (7)
RSOL _{SF} (mg COD gTVS ⁻¹ day ⁻¹)	27.1 (7)	49.9 (10)	61.6 (13)	59.9 (7)
RSOL _{ST} (mg COD gTVS ⁻¹ day ⁻¹)	26.7 (7)	49.6 (10)	57.7 (13)	56.1 (7)
V_{CH4} (N mL)	40.0 (5)	99.0 (5)	171 (5)	108 (5)
$Y_{CH4/COD}$ (N mL CH ₄ g COD ⁻¹)	29.5 (5)	39.6 (5)	55.5 (5)	36.0 (5)
%CH ₄ - ⁹ CO ₂	72–28 (5)	64–36 (5)	57–43 (5)	51–49 (5)

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

Biomass present in the reactor (average value for the four conditions)

S'_{TS} =1,543±58 mg TS g foam⁻¹; S'_{TVS} =1,261±45 mg TVS g foam⁻¹

S_{TS} =61±1 g TS L⁻¹; S_{TVS} =50±1 g TVS L⁻¹; S_{TVS}/S_{TS} =0.82 g TVS g TS⁻¹

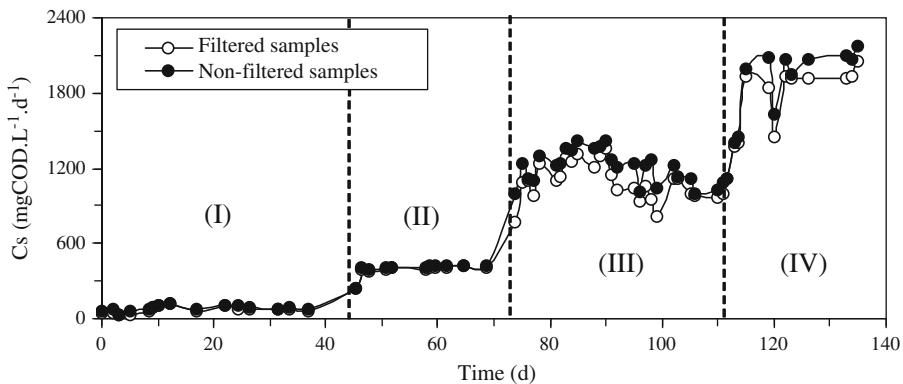


Fig. 2 Effluent organic matter concentration during reactor operation [(I) $AVOL=1.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (II) $AVOL=3.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (III) $AVOL=4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (IV) $AVOL=6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$]

buffer the medium and maintain reactor stability at all the imposed conditions. At higher AVOLs, the concentration of intermediate volatile acids increased. The main intermediate volatile acids identified were acetic acid (maximum of 240 and 320 mg L^{-1} at the third and fourth operational conditions, respectively) and propionic acid (maximum of 215, 500, and 950 mg L^{-1} at the second, third, and fourth operational conditions, respectively), followed by valeric and isovaleric acid present at lower concentrations (maximum of 3.44 mg L^{-1} at all conditions).

Profiles of volumetric production of methane, depicted in Fig. 9a, showed methane formation over the whole course of the cycle at the four investigated conditions, thus confirming the results on organic matter removal efficiency and demonstrating that the reactor was not simply retaining or accumulating organic matter, but indeed degrading the substrate. At AVOLs of 1.5, 3.0, 4.5, and 6.0 $\text{gCOD L}^{-1} \text{ day}^{-1}$, total methane production per cycle was 40, 99, 171, and 108 mL, respectively, (Table 2), i.e., total volumetric methane production increased with increasing AVOL from 1.5 to 4.5 $\text{gCOD L}^{-1} \text{ day}^{-1}$. On increasing AVOL from 4.5 to 6.0 $\text{gCOD L}^{-1} \text{ day}^{-1}$, total methane production dropped, which may be related to the increase in maximum concentration of total volatile acids along the cycle, which were 482 and 740 mg HAc L^{-1} , respectively (Figs. 7 and 8). This fact

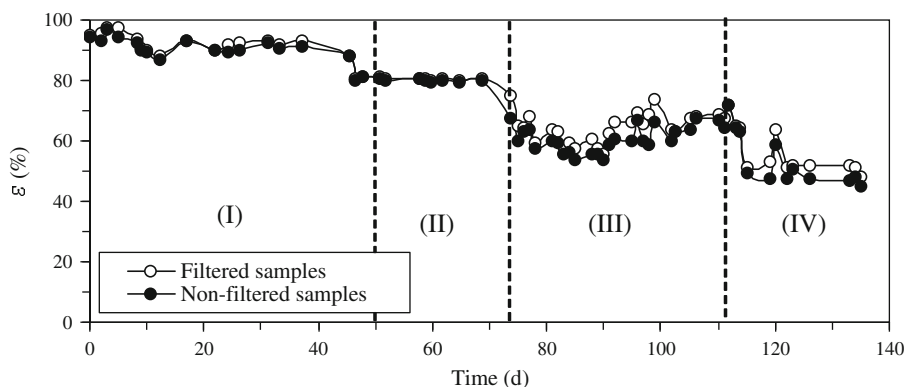


Fig. 3 Organic matter removal efficiency during reactor operation [(I) $AVOL=1.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (II) $AVOL=3.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (III) $AVOL=4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (IV) $AVOL=6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$]

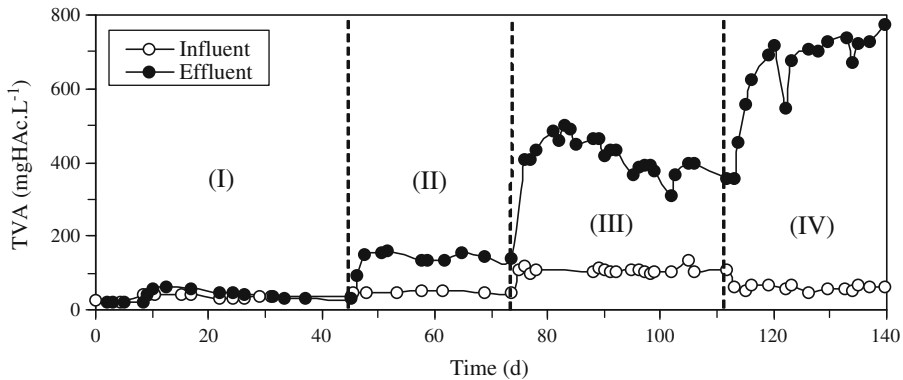


Fig. 4 Total volatile acids concentration during reactor operation [(I) $AVOL=1.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (II) $AVOL=3.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (III) $AVOL=4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$; (IV) $AVOL=6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$]

demonstrates that the same change may have occurred in the anaerobic biomass metabolism, resulting in lower production of methane. In the same way, Fig. 9b shows an increase in the relation between volumetric production of methane and the mass of removed organic matter per cycle (29.5, 39.6, and 55.5 $\text{mL CH}_4\text{g COD}^{-1}$ at AVOLs of 1.5, 3.0, and 4.5 $\text{gCOD L}^{-1} \text{ day}^{-1}$, respectively) and a drop to 36.0 $\text{mL CH}_4\text{g COD}^{-1}$ when AVOL was increased from 4.5 to 6.0 $\text{gCOD L}^{-1} \text{ day}^{-1}$.

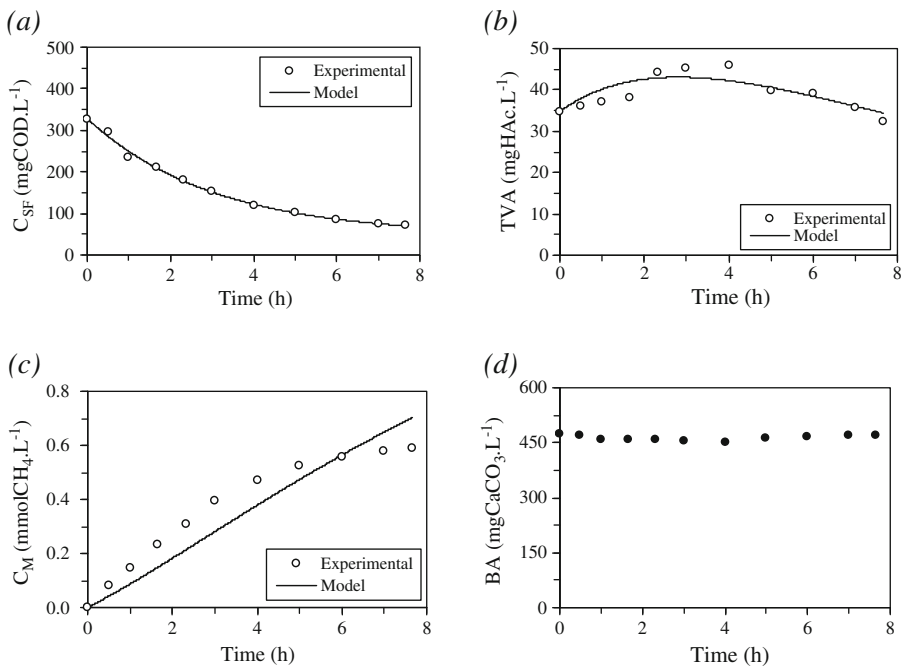


Fig. 5 Time profiles of **a** organic matter total, **b** volatile acids, **c** methane and **d** bicarbonate alkalinity for the condition at which $AVOL=1.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$

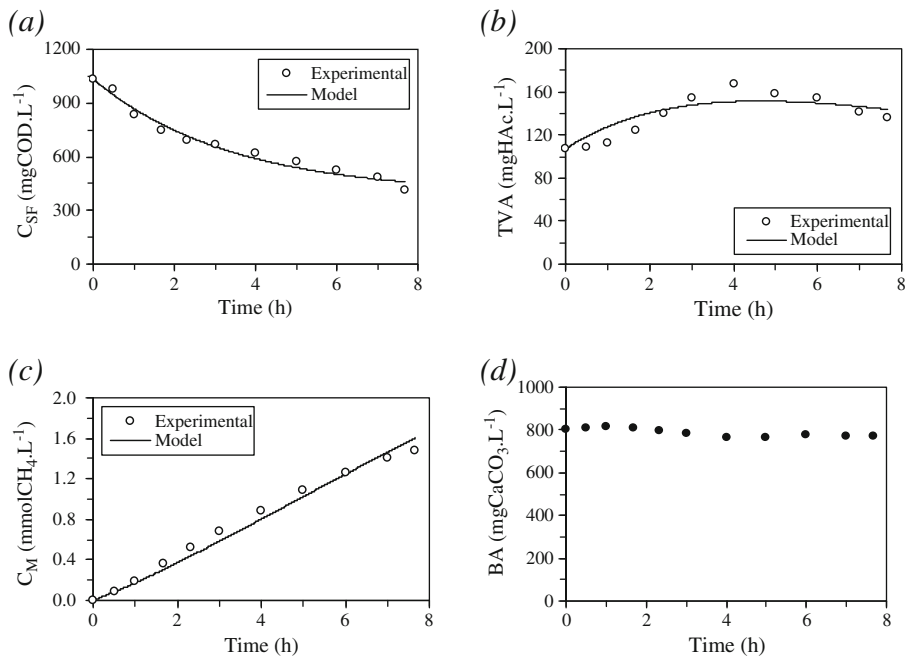


Fig. 6 Time profiles of **a** organic matter total, **b** volatile acids, **c** methane and **d** bicarbonate alkalinity for the condition at which $AVOL = 3.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$

The ratio between methane produced and organic matter consumed (i.e., methane yield) is better understood taking into account the removed (volumetric or specific) organic load instead of organic matter efficiency, which presents direct behavior, as shown in Table 2.

The CH_4 content decreased as $AVOL$ increased due to the amount of NaHCO_3 supplemented to the influent, resulting in an increase in CO_2 release.

Selma et al. [31] also investigated the production of methane by anaerobic conversion of the same effluent used in this work, employing a mechanically stirred ASBR containing granular biomass, at different feed strategies (10 min batch, 2- and 4-h fed-batch) and organic loadings ($AVOL$ of 0.60, 1.29, 2.44, and $3.82 \text{ gCOD L}^{-1} \text{ day}^{-1}$) in 8-h cycles. Ratios between produced methane volume and consumed organic matter per cycle ($Y_{\text{CH}_4/\text{COD}}$; 95.5, 83.8, and $78.1 \text{ mL CH}_4 \text{ g COD}^{-1}$, at $AVOL$ s of 0.60, 1.29, and $2.44 \text{ g COD L}^{-1} \text{ day}^{-1}$, respectively) exceeded those encountered in the current investigation, indicating that biomass immobilization in polyurethane foam impairs biogas release, resulting in lower $Y_{\text{CH}_4/\text{COD}}$ values.

Table 2 and Fig. 10a and b show, respectively, the values of removed volumetric organic load (RVOL) and removed specific organic load (RSOL) as a function of $AVOL$. RVOL increased with increasing $AVOL$ of 1.5 to $4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$ for filtered (1.36, 2.49, and $3.08 \text{ gCOD L}^{-1} \text{ day}^{-1}$ at $AVOL$ of 1.5, 3.0, and $4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$, respectively), as well as for unfiltered effluent samples (1.34, 2.48, and $2.88 \text{ gCOD L}^{-1} \text{ day}^{-1}$ at $AVOL$ of 1.5, 3.0, and $4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$, respectively) and a slight drop when loading increased to $6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$ (3.00 and $2.81 \text{ gCOD L}^{-1} \text{ day}^{-1}$ for filtered and unfiltered effluent samples, respectively). This drop in removed organic matter might be related to the increase in the maximum of total volatile acids along the cycle when $AVOL$ increased from 4.5 to $6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$. The same behavior was observed in terms of removed specific

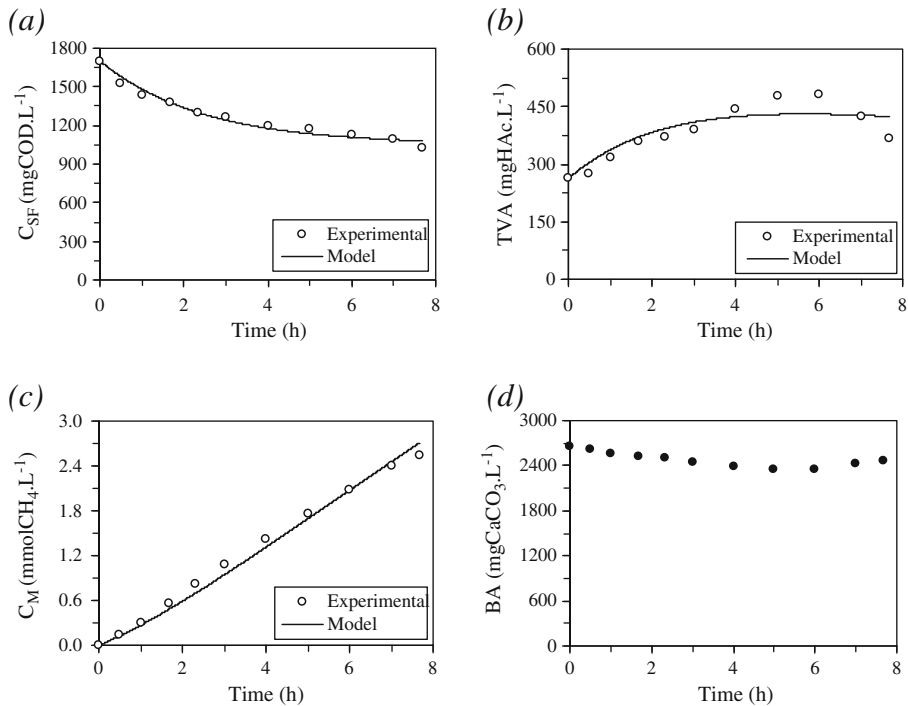


Fig. 7 Time profiles of **a** organic matter total, **b** volatile acids, **c** methane and **d** bicarbonate alkalinity for the condition at which $AVOL = 4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$

organic load, which increased when $AVOL$ increased from 1.5 to $4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$ for filtered effluent samples (27.1, 49.9, and $61.6 \text{ mg COD gTVS}^{-1} \text{ day}^{-1}$ at $AVOL$ s of 1.5, 3.0, and $4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$, respectively), as well as for unfiltered ones ($26.7, 49.6$ and $57.7 \text{ mg COD gTVS}^{-1} \text{ day}^{-1}$ at $AVOL$ s of 1.5, 3.0, and $4.5 \text{ gCOD L}^{-1} \text{ day}^{-1}$, respectively), with a slight drop when loading was increased to $6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$ (59.9 and $56.1 \text{ mg COD gTVS}^{-1} \text{ day}^{-1}$ for filtered and unfiltered effluent samples, respectively).

Comparison of these results with those of Selma et al. [31] shows poorer performance of the ASBR in which $RVOL$ values were 0.57, 1.10, and $1.78 \text{ gCOD L}^{-1} \text{ day}^{-1}$ and $RSOL$ 14.5, 28.0, and $45.3 \text{ mg COD gTVS}^{-1} \text{ day}^{-1}$ for filtered effluent samples at $AVOL$ s of 0.60, 1.29, and $2.44 \text{ gCOD L}^{-1} \text{ day}^{-1}$ which, in terms of $ASOL$, corresponded to 15.2, 32.8, and $62.1 \text{ mg COD gTVS}^{-1} \text{ day}^{-1}$, respectively. Organic matter removal efficiencies obtained in their work were 95%, 85%, and 73% for filtered effluent samples and 93%, 81%, and 66%, for unfiltered effluent samples at $AVOL$ s of 0.60, 1.29, and $2.44 \text{ gCOD L}^{-1} \text{ day}^{-1}$, respectively, i.e., lower than those obtained in the current investigation. Hence, despite the lower production of methane compared to the mechanically stirred reactor containing granular biomass (ASBR), the reactor employed in the current investigation using immobilized biomass and mixing by recirculation of the liquid phase (AnSBBR), showed to be more efficient in terms of organic matter removal. It should also be mentioned that the ASBR did not attain stability at $AVOL$ of $3.82 \text{ gCOD L}^{-1} \text{ day}^{-1}$, whereas the AnSBBR maintained stability up to $6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$.

The bioreactor used in the experiments contained biomass immobilized on polyurethane foam as inert support. The use of this support results in non-significant discharged solids, as

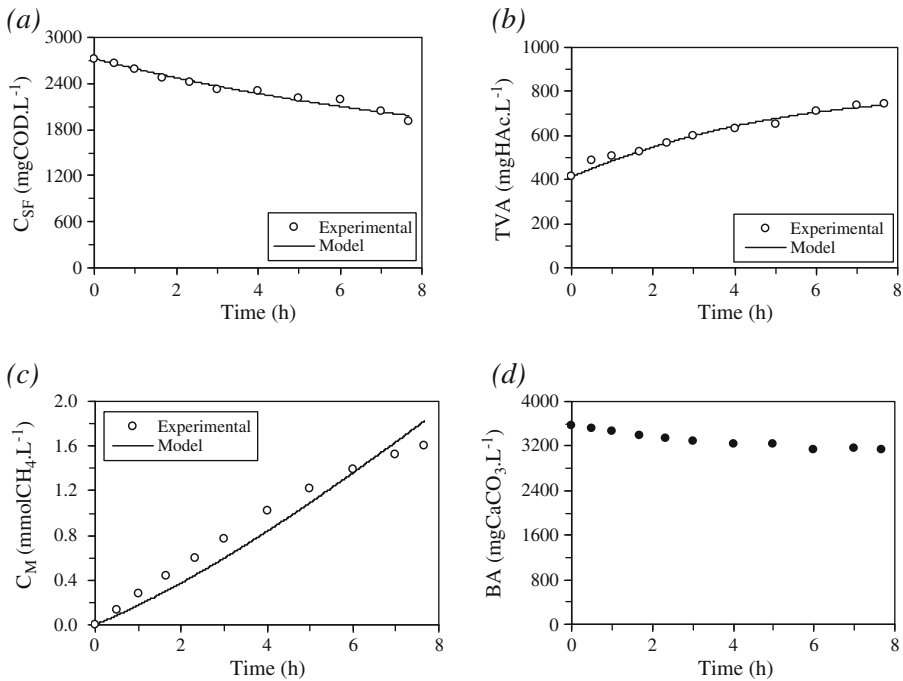


Fig. 8 Time profiles of **a** organic matter total, **b** volatile acids, **c** methane and **d** bicarbonate alkalinity for the condition at which $AVOL = 6.0 \text{ gCOD L}^{-1} \text{ day}^{-1}$

shown in TS/TVS/TSS/VSS analysis values and standard deviation for the influent and effluent (Tables 1 and 2). This fact does not allow precise SRTs estimation for the different implemented AVOLs.

Table 3 lists the values of the parameters obtained from fitting the first-order kinetic model to the experimental data of the following concentration profiles: filtered organic matter, total volatile acids and methane in the liquid phase for the four investigated AVOLs. The kinetic parameter relative to the consumption of filtered organic matter (k_{1S}) was approximately 0.32 and 0.31 h⁻¹ at AVOLs of 1.5 and 3.0 gCOD L⁻¹ day⁻¹, respectively. This shows that for the first two operation conditions, variation in applied AVOL did not

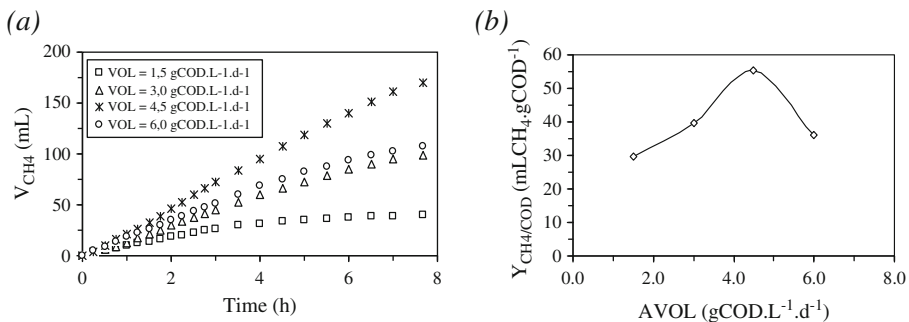


Fig. 9 Volumetric methane production time profiles **(a)** and ratio of volumetric methane production to removed organic matter mass **(b)** as a function of the applied volumetric organic load

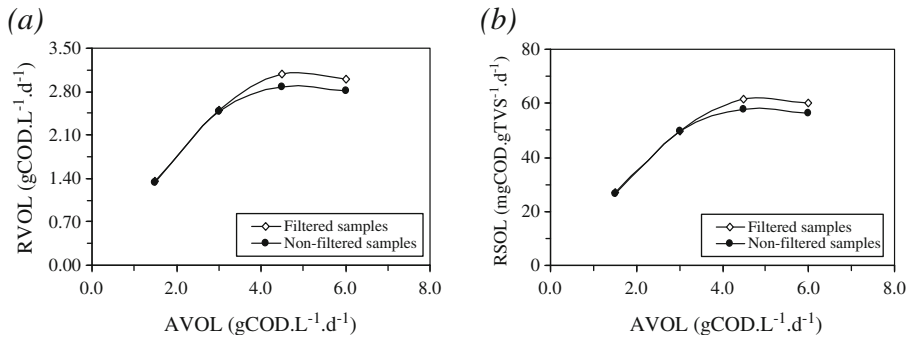


Fig. 10 Removed volumetric organic load (a) and removed specific organic load (b) as a function of the applied volumetric organic load

cause significant changes in the consumption rate of filtered organic matter. Increasing AVOL from 1.5 to 3.0 gCOD L⁻¹ day⁻¹ resulted in increase in residual concentration of filtered organic matter (C_{SR}) from 47.7 to 394 mg COD L⁻¹, whereas removal efficiency of organic matter dropped. At the third operational condition when AVOL was increased to 4.5 gCOD L⁻¹ day⁻¹ a significant increase was observed in the kinetic parameter relative to the consumption of filtered organic matter (k_{1S}) from 0.31 to 0.41 h⁻¹. However, at this condition, the residual concentration of filtered organic matter (C_{SR}) also increased to 1,053 mg COD L⁻¹, indicating that despite the higher consumption rate of organic matter in relation to the two previous conditions (hence, allowing reactor operation at shorter cycle length) filtered organic matter concentration in the effluent increased in relation to the previous conditions. Increasing AVOL to 6.0 gCOD L⁻¹ day⁻¹ caused a significant drop in k_{1S} to 0.10 h⁻¹, indicating a decrease in organic matter consumption rate. Simultaneously, the residual concentration of filtered organic matter (C_{SR}) increased to 1,345 mg COD L⁻¹, reducing reaction rate. It should be mentioned that the k_{1S} values estimated by Selma et al. [31] employing a mechanically stirred ASBR containing granular biomass (0.62, 0.20, and 0.24 h⁻¹, at AVOLs of 0.60, 1.29, and 2.44 gCOD L⁻¹ day⁻¹, respectively) were lower than those verified in this work, corroborating the interpretation related to the improved ability

Table 3 Estimated first-order kinetic parameters for the four applied volumetric organic load operational conditions

Variable	AVOL (g COD L ⁻¹ day ⁻¹)			
	1.5	3.0	4.5	6.0
k_{1S} (h ⁻¹)	0.32	0.31	0.41	0.10
k_{1TVA} (h ⁻¹)	0.04	0.05	0.15	0.07
k_{2TVA} (h ⁻¹)	0.10	0.05	0.02	0.04
k_{2M} (h ⁻¹)	0.00	0.00	0.00	0.00
C_{SR} (mg COD L ⁻¹)	47.7	394	1,053	1,345
C_{TVAR} (mg HAc L ⁻¹)	0.00	0.00	0.00	0.00
r^2	0.944	0.966	0.964	0.987
k_{1S}/k_{1TVA}	8.2	6.0	2.7	1.4
k_{2M}/k_{2TVA}	0.0	0.0	0.0	0.0

of organic matter consumption by the AnSBBR in relation to the conventional ASBR. The behavior of the parameter k_{1S} as a function of applied AVOL is illustrated in Fig. 11a.

The parameter k_{1S}/k_{1AVT} , which may be considered the stoichiometric relation a/b in Eq. 2, showed a practically constant drop as AVOL increased: 8.2, 6.0, 2.7, and 1.4 h^{-1} at AVOL of 1.5, 3.0, 4.5, and 6.0 $\text{gCOD L}^{-1} \text{ day}^{-1}$, respectively, as listed in Table 3 and illustrated in Fig. 11b. The drop in the relation k_{1S}/k_{1AVT} indicates a possible change in the metabolic routes of the process, so that the number of moles of substrate required to form 1 mol total volatile acids decreased with increasing AVOL. This observation is coherent with the experimental data obtained, which showed higher TVA concentrations along the cycle at higher AVOL. Analysis of the parameters k_{2TVA} , k_{2M} , C_{TVA} , and k_{2M}/k_{2TVA} was impaired because of the low values obtained of these parameters (Table 3), of which the profiles were practically constant and did not show any significant trend.

Conclusions

This work showed that increasing applied volumetric organic load resulted in a drop of the organic matter removal efficiency and increase in total volatile acids concentration of the reactor effluent. Despite this increase, no acids accumulated over time. High acid concentrations in the effluent occurred because cycle length was insufficient for the consumption of these acids at organic loading rates ranging from 3.0 to 6.0 $\text{g COD L}^{-1} \text{ day}^{-1}$.

Increasing the applied volumetric organic load from 1.5 to 4.5 $\text{gCOD L}^{-1} \text{ day}^{-1}$ resulted in increase in the following parameters: methane production, removed volumetric organic load, removed specific organic load, and the kinetic parameter value of filtered organic matter consumption. However, on increasing the applied volumetric organic load from 4.5 to 6.0 $\text{g COD L}^{-1} \text{ day}^{-1}$, these parameters dropped; likely due to the increase in the maximum total volatile acids concentration along the cycle, which may have caused by intermediate compounds accumulation, suggesting that for this specific reactor configuration and wastewater, the maximum applied volumetric organic load limit to be applied is 6.0 $\text{g COD L}^{-1} \text{ day}^{-1}$. Despite the drop in methane production and the higher concentration of volatile acids at the highest applied volumetric organic load of 6.0 $\text{gCOD L}^{-1} \text{ day}^{-1}$, alkalinity

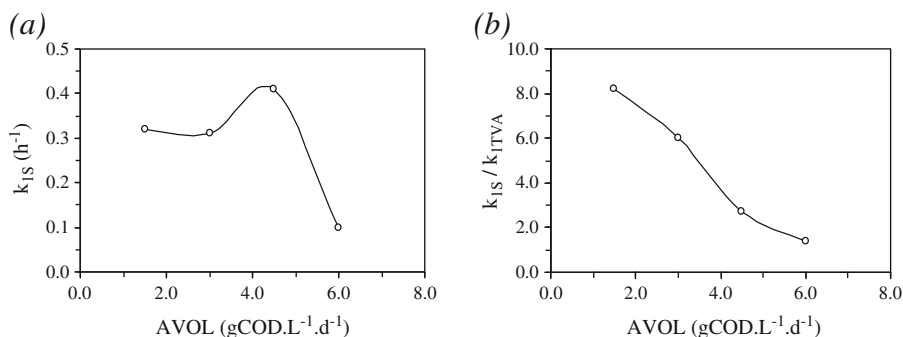


Fig. 11 Apparent kinetic parameter associated with the substrate consumption (a) and ratio of apparent kinetic parameter associated with the substrate consumption to that associated with the total volatile acids formation (b) as a function of the applied volumetric organic load

supplementation to the influent, in the form of sodium bicarbonate, was sufficient to maintain pH of the medium close to seven and guarantee process stability during reactor operation.

Despite the lower production of methane by the AnSBBR, using immobilized biomass and mixing by recirculation of the liquid phase, compared to the mechanically stirred ASBR containing granular biomass, the former showed improved ability to remove volumetric and specific organic load as well as improved organic matter consumption rate.

In general, the system showed to be robust and responded well to the increase in applied volumetric organic loading, i.e., despite deficiency in alkalinity presented by the effluent of the biodiesel production process, the reactor enabled organic matter removal as well as methane production. The possibility to obtain, recover and eventually utilize the methane generated during the treatment may render the biofuel production process economically more feasible.

Notation

BA	Bicarbonate alkalinity, mg $\text{CaCO}_3 \text{L}^{-1}$
C_{CH_4}	Methane concentration in the gas phase, mmol L^{-1}
C_{CO_2}	Carbonic gas concentration in the gas phase, mmol L^{-1}
C_{M}	Methane concentration in the liquid phase in the reactor, mmol $\text{CH}_4 \text{L reaction volume}^{-1}$
C_{SF}	Organic matter concentration for filtered samples, mg COD L^{-1}
C_{SR}	Residual organic matter concentration for filtered samples, mg COD L^{-1}
C_{ST}	Organic matter concentration for non-filtered effluent samples, mg COD L^{-1}
C_{TS}	Total solids concentration, mg TS L^{-1}
C_{TSS}	Total suspended solids concentration, mg TSS L^{-1}
C_{TVA}	Total volatile acids concentration, mg HAc L^{-1}
C_{TVA0}	Total volatile acids concentration in the influent, mg HAc L^{-1}
C_{TVAR}	Residual total volatile acids concentration in the reactor, mg HAc L^{-1}
C_{TVS}	Total volatile solids concentration, mg TVS L^{-1}
C_{VSS}	Volatile suspended solids concentration, mg VSS L^{-1}
$k_{1\text{S}}$	First-order apparent kinetic parameter associated with the substrate consumption, h^{-1}
$k_{1\text{TVA}}$	First-order apparent kinetic parameter associated with the total volatile acids formation, h^{-1}
$k_{2\text{M}}$	First-order apparent kinetic parameter associated with the methane formation, h^{-1}
$k_{2\text{TVA}}$	First-order apparent kinetic parameter associated with the total volatile acids consumption, h^{-1}
M_{TVS}	Total volatile solids mass in the reactor, g TVS
r_{M}	Methane formation rate, mg $\text{CH}_4 \text{L}^{-1} \text{day}^{-1}$
r_{S}	Substrate consumption reaction rate, mg COD $\text{L}^{-1} \text{h}^{-1}$
r_{TVA}	Total volatile acids formation and consumption rate, mg HAc $\text{L}^{-1} \text{h}^{-1}$
S'_{TS}	Total solids concentration relative to the immobilized biomass, mg TS gfoam $^{-1}$
S'_{TVS}	Total volatile solids concentration relative to the immobilized biomass, mg TVS gfoam $^{-1}$
S_{TS}	Total solids concentration relative to the immobilized biomass, mg TS L reaction volume $^{-1}$

S_{TVS}	Total volatile solids concentration relative to the immobilized biomass, mg TVS L reaction volume ⁻¹
S_{TVS}/S_{TS}	Total volatile solids concentration to total solids concentration ratio in the reactor, g TVS g TS ⁻¹
t	Time during a cycle, h
t_c	Cycle time, h
V	Reaction medium volume of the system, L
V_A	Fed volume or renewed volume per cycle, L
V_{CH_4}	Normal volumetric methane production per cycle, mL CH ₄
$Y_{CH_4/COD}$	Ratio of volumetric methane production to removed organic matter mass, mL CH ₄ g COD ⁻¹
AVOL	Applied volumetric organic load, g COD L ⁻¹ day ⁻¹
ASOL	Applied specific organic load, mg COD gTVS ⁻¹ day ⁻¹
RSOL _{SF}	Removed specific organic load for filtered effluent samples, mg COD gTVS ⁻¹ day ⁻¹
RSOL _{ST}	Removed specific organic load for non-filtered effluent samples, mg COD gTVS ⁻¹ day ⁻¹
RVOL _{SF}	Removed volumetric organic load for filtered effluent samples, g COD L ⁻¹ day ⁻¹
RVOL _{ST}	Removed volumetric organic load for non-filtered effluent samples, g COD L ⁻¹ day ⁻¹
%CH ₄	Methane molar percentage in the gas phase, %
%CO ₂	Carbonic gas molar percentage in the gas phase, %
ε	Organic matter removal efficiency, %

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References

1. Angenent, L. T., Sung, S., & Raskin, L. (2002). Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. *Water Research*, 36, 4648–4654.
2. Bagley, D. M., & Brodtkorb, T. S. (1999). Modeling microbial kinetics in an anaerobic sequencing batch reactor – model development and experimental validation. *Water Environmental Research*, 71, 1320–1332.
3. Bezerra, R. A., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., & Foresti, E. (2009). Effects of feed time, organic loading and shock loads in the anaerobic whey treatment by an AnSBBR with circulation. *Applied Biochemistry and Biotechnology*, 157, 140–158.
4. Borges, A. C., Siman, R. R., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., Foresti, E., et al. (2004). Stirred anaerobic sequencing batch reactor containing immobilized biomass: a behavior study when submitted to different fill times. *Water Science and Technology*, 49, 311–318.
5. Bouallagui, H., Lahdheb, H., Ben Romdan, E., Rachdi, B., & Hamdi, M. (2009). Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. *Journal of Environmental Management*, 90, 1844–1849.
6. Brito, A. G., Rodrigues, A. C., & Melo, F. L. (1997). Feasibility of a pulsed sequencing batch reactor with an anaerobic aggregated biomass for the treatment of low strength wastewaters. *Water Science and Technology*, 35, 193–198.
7. Camargo, E. F. M., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., & Borzani, W. (2002). Treatment of low-strength wastewater using immobilized biomass in a sequencing batch external loop reactor:

- influence of the medium superficial velocity on the stability and performance. *Brazilian Journal of Chemical Engineering*, 19, 267–275.
8. Canto, C. S. A., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., & Foresti, E. (2008). Anaerobic Sequencing Batch Biofilm Reactor (AnSBBR): Some applications to sanitary and industrial wastewaters. (Chapter 2). In R. H. Theobald (Ed.), *Environmental management* (p. 429 p). New York: Nova.
 9. Canto, C. S. A., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., & Foresti, E. (2008). Feasibility of nitrification/denitrification in a sequential batch biofilm reactor with liquid circulation applied to post-treatment. *Bioresource Technology*, 99, 644–654.
 10. Chebel, F. X., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., & Foresti, E. (2006). Analysis of performance of an anaerobic sequential batch reactor submitted to increasing organic load with different influent concentrations and cycle lengths. *Applied Biochemistry and Biotechnology*, 133, 171–187.
 11. Damasceno, L. H. S., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., & Foresti, E. (2007). Effects of feeding time and organic loading in an anaerobic sequencing batch biofilm reactor (ASBBR) treating diluted whey. *Journal of Environmental Management*, 85, 927–935.
 12. Damasceno, L. H. S., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., & Foresti, E. (2008). Effect of mixing mode on the behavior of an ASBBR with immobilized biomass in the treatment of cheese whey. *Brazilian Journal of Chemical Engineering*, 25, 291–298.
 13. Dilallo, R., & Albertson, O. E. (1961). Volatile acids by direct titration. *Journal Water Pollution Control Federation*, 3, 356–365.
 14. Ito, T., Nakashimada, Y., Senba, K., Matsui, T., & Nishio, N. (2005). Hydrogen and ethanol production from glycerol containing wastes discharges after biodiesel manufacturing process. *Journal of Bioscience and Bioengineering*, 100, 260–265.
 15. Lehninger, A. L., Nelson, D. L., & Cox, M. M. (1993). *Principles of biochemistry* (2nd ed.). New York: Worth Publishers.
 16. Massé, D. I., & Masse, L. (2000). Treatment of slaughterhouse wastewater in anaerobic sequencing batch reactors. *Canadian Agricultural Engineering*, 42, 131–137.
 17. Michelan, R., Zimmer, T. R., Rodrigues, J. A. D., Ratusznei, S. M., Moraes, D., Zaiat, M., et al. (2009). Effect of impeller type and mechanical agitation on the mass transfer and power consumption aspects of ASBR operation treating synthetic wastewater. *Journal of Environmental Management*, 90, 1357–1364.
 18. Moreira, M. B., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., & Foresti, E. (2008). Influence of organic shock loads in an ASBBR treating synthetic wastewater with different concentration levels. *Bioresource Technology*, 99, 3256–3266.
 19. Ndegwa, P. M., Hamilton, D. W., Lalman, J. A., & Cumba, H. J. (2005). Optimization of anaerobic sequencing batch reactors treating dilute swine slurries. *Transactions of the ASAE*, 48, 1575–1583.
 20. Nishio, N., & Nakashimada, Y. (2007). Recent development of digestion process for energy recovery from wastes. *Journal of Bioscience and Bioengineering*, 103, 105–112.
 21. Oliveira, R. P., Ghilardi, J. A., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., & Foresti, E. (2008). Anaerobic sequencing batch biofilm reactor applied to automobile industry wastewater treatment: volumetric loading rate and feed strategy effects. *Chemical Engineering and Processing*, 47, 1380–1389.
 22. Oliveira, D. S., Prinholato, A. C., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., & Foresti, E. (2009). AnSBBR applied to the treatment of wastewater from a personal care industry: effect of organic load and fill time. *Journal of Environmental Management*, 90, 3070–3081.
 23. Orra, A. A., Ratusznei, S. M., Rodrigues, J. A. D., Foresti, E., & Zaiat, M. (2004). Effects of feeding strategies on the performance of an anaerobic discontinuous reactor containing immobilized biomass with circulation system for liquid-phase mixing. *Water Science and Technology*, 49, 303–310.
 24. Perry, R. H., & Green, D. W. (1997). *Chemical engineers' handbook* (7th ed.). New York: McGraw-Hill.
 25. Ramos, A. C. T., Ratusznei, S. M., Rodrigues, J. A. D., & Zaiat, M. (2003). Mass transfer improvement of a fixed-bed anaerobic sequencing batch reactor with liquid phase circulation. *Journal of Science and Technology of the Americas – Interciencia*, 28, 214–219.
 26. Ripley, L. E., Boyle, W. C., & Converse, J. C. (1986). Improved alkalimetric monitoring for anaerobic digester of high-strength wastes. *Journal Water Pollution Control Federation*, 58, 406–411.
 27. Rodrigues, J. A. D., Pinto, A. G., Ratusznei, S. M., Zaiat, M., & Gedraite, R. (2004). Enhancement of the performance of an anaerobic sequencing batch reactor treating low strength wastewater through implementation of a variable stirring rate program. *Brazilian Journal of Chemical Engineering*, 21, 423–434.
 28. Rodrigues, J. A. D., Ratusznei, S. M., Camargo, E. F. M., & Zaiat, M. (2003). Influence of agitation rate on the performance of an anaerobic sequencing batch reactor containing granulated biomass treating low-strength wastewater. *Advances in Environmental Research*, 7, 405–410.
 29. Sabourin-Provost, G., & Hallenbeck, P. C. (2009). High yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by photofermentation. *Bioresource Technology*, 100, 3513–3517.

30. Sahinkaya, E., & Dilek, F. B. (2007). Effect of feeding time on the performance of a sequencing batch reactor treating a mixture of 4-CP and 2,4-DCP. *Journal of Environmental Management*, 83, 427–436.
31. Selma, V. C., Cotrim, L. H. B., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., & Foresti, E. (2010). ASBR applied to the treatment of biodiesel production effluent: effect of organic load and fill time on performance and methane production. *Applied Biochemistry and Biotechnology*, 162, 2365–2380.
32. Sirianuntapiboon, S., Chairattananaw, K., & Surasinanant, P. (2007). Some properties of a sequencing batch reactor for treatment of wastewater containing thiocyanate compounds. *Journal of Environmental Management*, 85, 330–337.
33. Sirianuntapiboon, S., Sadahiro, O., & Salee, P. (2007). Some properties of a granular activated carbon-sequencing batch reactor (GAC-SBR) system for treatment of textile wastewater containing direct dyes. *Journal of Environmental Management*, 85, 162–170.
34. Standard Methods for the Examination of Water and Wastewater. (1995). *APHA, AWWA, WPCF* (19th ed.). Washington: American Public Health Association.
35. Suehara, K., Kawamoto, Y., Fujii, E., Kohda, J., Nakano, Y., & Yano, T. (2005). Biological treatment of wastewater discharged from biodiesel fuel production plant with alkali-catalyzed transesterification. *Journal of Bioscience and Bioengineering*, 100, 437–442.
36. Varesche, M. B., Zaiat, M., Vieira, L. G. T., Vazoller, R. F., & Foresti, E. (1997). Microbial colonization of polyurethane foam matrices in horizontal-flow anaerobic immobilized-sludge reactor. *Applied Microbiology and Biotechnology*, 48, 534–538.
37. Xiangwen, S., Dangcong, P., Zhaohua, T., & Xinghua, J. (2008). Treatment of brewery wastewater using anaerobic sequencing batch reactor (ASBR). *Bioresource Technology*, 99, 3182–3186.
38. Yang, Y., Tsukahara, K., & Sawayama, S. (2008). Biodegradation and methane production from glycerol-containing synthetic wastes with fixed-bed bioreactor under mesophilic and thermophilic anaerobic conditions. *Process Biochemistry*, 43, 362–367.
39. Yazdani, S. S., & Gonzalez, R. (2007). Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. *Biotechnology*, 18, 213–219.
40. Zaiat, M., Cabral, A. K. A., & Foresti, E. (1994). Horizontal-flow anaerobic immobilized sludge reactor for wastewater treatment: conception and performance evaluation. *Revista Brasileira de Engenharia – Caderno de Engenharia Química*, 11, 33–42.
41. Zaiat, M., Rodrigues, J. A. D., Ratusznei, S. M., Camargo, E. F. M., & Borzani, W. (2001). Anaerobic sequencing batch reactors for wastewater treatment: a developing technology. *Applied Microbiology and Biotechnology*, 55, 29–35.