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ASBR Applied to the Treatment of Biodiesel Production Effluent: Effect of Organic Load and Fill Time on Performance and Methane Production

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Abstract The effect of organic matter and fill time on anaerobic sequencing batch reactor (5 L, 30°C, 8-h cycles, 50 rpm) efficiency has been analyzed. Organic matter was increased by the influent concentration. Fill times investigated were in the batch mode and fed-batch followed by batch. In the batch mode organic matter removal were 93%, 81%, and 66% for influent concentration of 500, 1,000, and 2,000 mgCOD/L (0.6, 1.29, and 2.44 gCOD/L.d), respectively. At 3,000 mgCOD/L (3.82 gCOD/L.d) operational stability could not be achieved. Removal efficiency was improved by increasing the fill time, and was 85% for the 1,000 mgCOD/L condition and fill times of 2 and 4 h, and 80 and 77% for the 2,000 mgCOD/L condition and fill times of 2 and 4 h, respectively. Hence, gradual feeding seemed to improve and to smooth the profiles of organic matter and volatile acids along the cycle with 78 to 96 NmLCH_4/gCOD.

Keywords ASBR · Biodiesel effluent · Organic load · Fill time · Methane

Introduction

The purpose of developing anaerobic sequencing batch reactor (ASBR) configurations has been to improve process performance and to make feasible its practical application. Projects focus on optimization of the bioreactor to make full-scale application feasible. A typical cycle of the anaerobic sequencing batch reactor includes four stages: (a) feed, which may have variable fill time; (b) treatment, through biotransformations of the wastewater

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constituents by microorganisms; (c) sedimentation, when biomass is granular (ASBR); when biomass is immobilized on inert support (AnSBBR), this stage is not necessary; and (d) discharge, by removing the treated and clarified liquid [1].

Advancements in the mechanically stirred reactor containing granular biomass show promising results achieved on bench and pilot scale [2, 3], applied to different wastewaters, such as sanitary, cheese whey, from the automotive and personal care industry, as well as effluents containing volatile acids, nitrogen, and sulfur compounds. Application of the anaerobic sequencing batch reactors containing granular biomass (ASBR) still depends on insight into some technological aspects, such as application to the treatment of industrial effluents to remove organic matter and obtain methane combined with the generation of the biogas produced in the biotransformation of pollutants.

Research on the application of this bioreactor configuration in the treatment of different effluents will contribute to better comprehension of the process. Many works are encountered in the literature on the influence of different process variables, which have as main objective the qualitative and quantitative knowledge of the relationship between these variables and efficiency and stability to optimize the system [4–6]. Studies on the influence of stirring, which is related to the mass transfer resistance from the liquid phase to biomass, affecting the global reaction rate, have been dealt with in a number of papers, whether by implementing liquid phase recirculation [7] or mechanical stirring [8–12]. Fill time is another important variable that has been investigated, and may influence process performance by the bioavailability of the substrate contained in the wastewater, by inhibition or toxicity of effluents with high organic load or lacking nutrients and/or alkalinity [13–15].

The abovementioned investigations evidence the importance of the fundamental studies on the behavior of mechanically stirred anaerobic sequencing batch reactors containing granular biomass [16]. However, another very important investigation line includes technological studies on such bioreactors, regarding system efficiency and stability when submitted to increasing organic loads in different wastewaters, such as synthetic wastewater [4, 5, 17], piggery effluents [8, 18], cheese whey effluents [14, 19], leachate [20], effluents from automotive industry [21], effluents from food and beverage plants [22, 23], effluents from personal care industry [24], and effluents of chemical industry [25–27].

Currently, works in the literature have shown an increase in the potential need for treatment of wastewaters from biofuel production processes. Particularly, the anaerobic biological treatment processes must be considered because in addition to the removal of organic matter they produce methane from the generated biogas and render the biofuel production process more advantageous in terms of cost effectiveness [28]. Works dealing with the anaerobic treatment of effluents from biodiesel production process include those of Ito et al. [29], Suehara et al. [30], Nishio and Nakashimada [31], Yang et al. [32], and Sabouring-Provost et al. [33].

Within this context, this investigation focuses on the study of the technological application of ASBR reactors in treating effluents generated in the transesterification reaction stage, followed by separation via sedimentation. At this stage the effluent contains as byproduct glycerin and the non-converted reagents soy oil and methanol. It should be mentioned that glycerin has commercial value as byproduct and is frequently used as such. However, the biodiesel production process generates 10 to 18 wt.% of raw glycerin. With the increasing production of biofuel expected for the next years, the large amount of glycerin generated will surpass the demand for this raw material by industries, such as cosmetics, for example. Thus, the importance of this paper is based on the potential need to treat this effluent, especially for small-scale biodiesel production units, and obtain methane from the generated biogas.

Therefore, the general purpose of this investigation was to assess the application of a mechanically stirred anaerobic sequencing batch reactor containing granular biomass (ASBR) in the treatment of effluents from the biodiesel production process in order to remove organic matter and produce methane. Reactor stability and efficiency were assessed as a function of increasing organic load applied to the system by varying the influent concentration as well as reactor fill time.

Materials and Methods

Experimental Setup

The mechanically stirred ASBR with useful volume of 5.0 L containing granular sludge was a BIOFLO III of New Brunswick Scientific Co with two six-vertical-flat-blade turbine impeller, as shown in Fig. 1. Feeding and discharge were performed by diaphragm pumps connected to automatic timers which provided for "on" and "off" switching of the pumps and the stirrer. The temperature was controlled at 30±1°C by means of a heating jacket fed by a thermostatic bath.

Inoculum

The inoculum used in the experiments came from an up-flow anaerobic sludge blanket reactor treating wastewater from a poultry slaughterhouse. This inoculum presented total solids (TS) and total volatile solids (TVS) of 62 and 51 g/L, respectively.

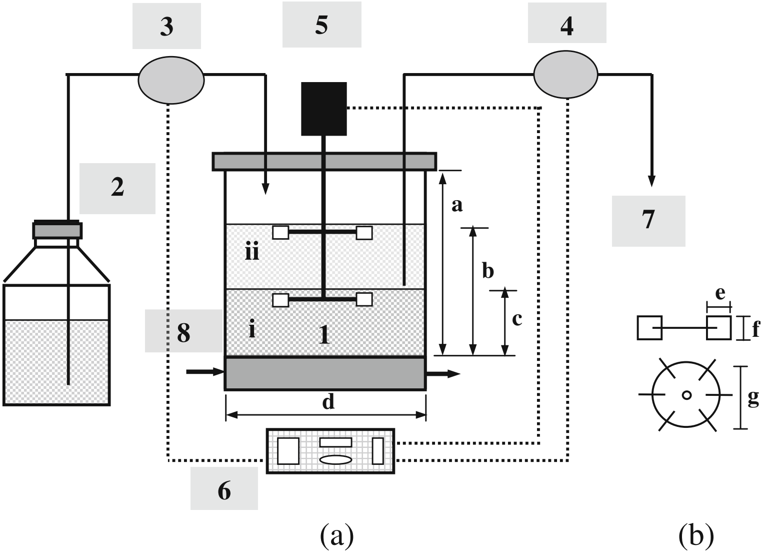


Fig. 1 Scheme of the mechanically stirred anaerobic sequencing batch biofilm reactor (ASBR). **a** Reactor [*1*—BIOFLO III Bioreactor (New Brunswick Scientific Co.), 5-L capacity (*a*=26 cm, *b* = 16 cm, *c*=8 cm, *d*=20 cm) and one turbine-type impeller with six flat blades (*e*=2 cm, *f*=1.5 cm, *g*=6 cm), containing granular biomass (*i*—high biomass concentration region, *ii*—low biomass concentration region); *2*—influent, *3*—feed pump, *4*—discharge pump, *5*—stirring system, *6*—automation system, *7*—effluent, *8*—temperature control via bottom jacket]. **b** Turbine-type impeller with six flat blades in detail

Wastewater

The wastewater used was prepared by diluting effluent from the biodiesel production process and considered only the production stage, i.e., obtained from the separation of formed biodiesel from the byproducts in the reaction stage, consisting primarily of glycerin, in addition to non-reacted soy oil and methanol. The biodiesel production procedure was carried out on laboratory scale from soy oil and methanol.

Analytical Methods

During reactor operation influent and effluent samples were taken for the following analyses: organic matter concentration of the filtered and unfiltered effluent as COD (C_{SF} and C_{ST}, respectively), organic matter concentration of the unfiltered influent as COD (C_I; spectrophotometric), total volatile acids (TVA—potentiometric) and intermediate volatile acids (IVA—chromatographic), bicarbonate alkalinity (potentiometric), TS and TVS, total suspended solids, and volatile suspended solids (gravimetric); and pH and volume fed per cycle (measured in a measuring cylinder). Methods employed were according to *Standard Methods for Examination of Water and Wastewater* [34].

IVA samples were analyzed by gas chromatography, using a gas chromatograph HP6890 with flame ionization detector at 300°C and an HP-INNOWAX column (30 m × 0.25 mm × 0.25 mm). The injector temperature was kept at 250°C; the oven was held at 100°C for 3 min, after which it was heated at a rate of $5°\mathrm{C\,min}^{-1}$ to 180°C and held at that temperature for 5 min.

The production of biogas generated by anaerobic degradation in the batch feeding operation was measured by an offset gas meter of NaOH solution (concentration 50 g/L) for CO_2 absorption. The gas meter was not used in the fed-batch feeding operation because of volume variations resulting from gradual feeding along the cycle. Composition of the biogas generated by anaerobic degradation was analyzed by gas chromatography using a Hewlett Packard® 6890 gas chromatograph equipped with thermal conductivity detector. The sample volume was 1 mL, drag gas was hydrogen at a flow rate of 50.0 mL/h, the column, injector, and detector temperatures were 35°C, 60°C, and 160°C, respectively.

At the end of operation condition biomass samples were withdrawn from the reactor for microbiological identification, which was performed by means of common optical and phase contrast microscopy employing an Olympus BH2® microscope.

Experimental Procedure

The experimental protocol used in this paper included ASBR reactor operation in sequencing batch (first stage) and fed-batch followed by sequencing batch (second stage). At all the investigated conditions the ASBR contained 2 L of anaerobic sludge and total liquid medium volume of 5 L. From this total volume, at every 8-h cycle (or 480 min), i.e., three cycles per day, 2 L of effluent were discharged, and 2 L of influent were fed afterwards. Thus, a residual volume of 1 L was maintained in the ASBR in each cycle. Stirring was performed at 50 rpm, using a mechanical stirrer with two three-blade impellers at 30±1°C. At all investigated conditions, the influent was supplemented with alkalinity by adding sodium bicarbonate ($NaHCO_3$).

In the *first stage* the ASBR was submitted to increasing influent concentrations to assess the effect of organic load on bioreactor efficiency and stability. Feeding lasted 10 min, thus characterizing batch operation, decant lasted 30 min and discharge 20 min, remaining thus 429 min for the react stage, since 1-min interval was allowed between the feed and discharge, as a safety factor in pump synchronization. The influent concentrations studied in the batch feeding condition were 500, 1,000, 2,000, and 3,000 mgCOD/L.

In the *second stage* the ASBR was submitted to different fill times, namely, 2 and 4 h, with influent concentrations of 1,000 and 2,000 mgCOD/L to assess the interaction between the effects of feed strategy and organic load on bioreactor efficiency and stability. The total volume of 5 L of liquid medium in the reactor and the volume fed and discharged of 2 L per 8-h cycle were maintained, as well as the 30-min sedimentation time, 10-min discharge time, and 50-rpm stirring. The objective of this stage was to check for potential improvement of the system efficiency with changes in reactor fill time.

Kinetic Model Formulation and Fitting

After reaching stability, verified by the attainment of approximately constant values for the monitored parameters of the effluent at final cycle condition, profiles were taken of filtered organic matter concentrations (C_S), bicarbonate alkalinity, total volatiles (C_{TVA}), intermediate acid, pH, and methane (C_M) and carbonic gas concentrations, and molar fractions. These profiles allowed better understanding of the organic matter degradation routes during an ASBR operation cycle, as well as estimation of kinetic parameters by fitting kinetic model to these profiles. Samples were taken at different time intervals during a cycle and the volume collected did not exceed 300 mL, being always 10% below total volume of wastewater in the reactor. After accomplishment of the profiles the condition was modified and a new operation phase was started.

The kinetic model used in this work was developed by Rodrigues et al. [10], based on the model of Bagley and Brodkorb [13]. 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 anaerobic sequencing batch reactor, the anaerobic process of organic matter degradation was simplified in two consecutive steps. In the first one the substrate (S) is converted into TVA, and in the second, these same acids are transformed into methane (M). Moreover, 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 1 to 4 present the organic matter degradation route and the reaction rate equations of substrate (R_S) consumption, formation of total volatile acids (R_{TVA}), and methane formation (R_M).

$$\mathrm{aS} \rightarrow \mathrm{b\ TVA} \rightarrow \mathrm{c\ M} \tag{1}$$

$$R_S = k_{1S} \cdot (C_S - C_{SR}) \tag{2}$$

$$R_{TVA} = k_{1TVA} \cdot (C_S - C_{SR}) - k_{2TVA} \cdot (C_{TVA} - C_{TVAR}) \tag{3}$$

$$R_M = k_{2M} \cdot (C_{TVA} - C_{TVAR}) \tag{4}$$

In Equation 1, letters a, b, and c stand for stoichiometric coefficients, and k_1 and k_2 stand for apparent kinetic parameters of the adopted model. In Eqs. 2, 3, and 4, k_{1S}, k_{1TVA}, k_{2TVA}, and k_{2M} are the same apparent kinetic parameters, but associated with the substrate consumption, total volatile acids formation, 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 reactor mass balance must consider two distinct operation conditions, being the first one in batch mode, and the second, in fed-batch mode. Equations 5, 6, and 7 present the mass balance of each matter concerning the kinetic model (substrate, total volatile acids, and methane), considering the operation in batch mode, whereas Eqs. 8, 9, and 10, refer to the same matters for the operation in fed-batch mode.

$$\frac{dC_{\mathrm{S}}}{dt} = -R_{\mathrm{S}} \tag{5}$$

$$\frac{dC_{\mathrm{TVA}}}{dt} = +R_{AVT} \tag{6}$$

$$\frac{dC_M}{dt} = +R_M \tag{7}$$

$$\frac{dC_{\mathrm{S}}}{dt} = \frac{F}{V} \cdot (C_{So} - C_S) - r_S \tag{8}$$

$$\frac{dC_{TVA}}{dt} = \frac{F}{V} \cdot (C_{TVAo} - C_{TVA}) + r_{TVA} \tag{9}$$

$$\frac{dC_{\mathrm{M}}}{dt} = -\frac{F}{V} \cdot C_M + r_M \tag{10}$$

In Eqs. 5 to 10 C_{S}, C_{TVA}, and C_{M} correspond to the concentrations of substrate, total volatile acid, and methane, respectively, and C_{So} and C_{TVAo}, the respective feed values, F is the volumetric flow rate, and V the reaction volume. These equations were used to determine the kinetic parameters $k_{1\mathrm{S}}$, $k_{1\mathrm{TVA}}$, $k_{2\mathrm{TVA}}$, $k_{2\mathrm{M}}$, C_{SR}, and C_{TVAR} of the model. To deal with the differential equations, Euler numerical integration method was used (constant integration step) implemented in Excel® software. Furthermore, 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 kinetic model data.

Results and Discussion

Effect of Organic Load in the Batch Operation

In the first phase (first four conditions) the ASBR was operated in batch mode (10-min feed time, i.e., fill time to total cycle time ratio of 0.02) to determine the maximum influent concentration, and consequently, the highest organic load (volumetric and/or specific) that could be applied to maintain system stability and efficiency (Tables 1, 2, 3, and 4).

Table 1 Mean values of monitored variables.

Condition	t_F/t_C	C_I (mgCOD/L)	C_S (mgCOD/L)		ε_S (%)		V (L)
			C_{ST}	C_{SF}	ε_{ST} (%)	ε_{SF} (%)	
B-500	0.02	492±16	37±9	24±5	93±2	95±1	2.02±0.11
B-1000	0.02	1,060±60	185±19	151±25	81±2	85±3	2.03±0.02
B-2000	0.02	1,996±135	681±74	547±61	66±4	73±3	2.04±0.05
B-3000	0.02	3,137±466	1,804±168	1,526±124	40±6	49±4	2.03±0.02
FB-2-1000	0.25	1,052±59	227±42	162±29	78±4	85±3	2.01±0.05
FB-4-1000	0.50	1,110±70	213±55	163±50	81±5	85±5	1.99±0.04
FB-2-2000	0.25	2,033±87	499±40	409±41	75±2	80±2	2.00±0.05
FB-4-2000	0.50	1,956±181	529±64	458±61	73±3	77±3	2.01±0.09

B batch operation, *FB* fed-batch operation (2–2 h, 4–4 h)

The *first operation condition* was 28 days (84 cycles) and 10-min fill time, with non-filtered influent concentration of 492±16 mgCOD/L, resulting in an applied volumetric organic load of 0.6 gCOD/L.d, with influent supplementation of 500 mg$NaHCO_3$/L. The system exhibited stable effluent values over a short period of time, i.e., the biomass was adapted to the operating conditions, obtaining removed volumetric organic load of 0.57 gCOD/L.d, for 95% removal efficiency in terms of filtered samples. Total volatile acid concentration was low, both in the influent (27 mgHAc/L) and in the effluent (32 mgHAc/L), and bicarbonate alkalinity was generated (influent 386 mg$CaCO_3$/L and effluent 431 mg$CaCO_3$/L). The volume of methane produced per cycle was 90.7 NmL for a concentration of 5.51 mmol/L and composition of 71% (assuming CH_4 and CO_2 only), i.e., for 5-L medium volume and 8-h cycle, the ratio between the volume of methane produced and the amount of organic matter removed was 95.5 NmLCH_4/gCOD, and the ratio between methane concentration and the difference of organic matter concentration (non-filtered influent and effluent) was 11.8 mmolCH_4/gCOD.

Table 2 Mean values of monitored variables.

Condition	R	BA		TVA		V_{CH4}	C_{CH4}
	$NaHCO_3$/COD	(mg$CaCO_3$/L)		(mgHAc/L)			
		Influent	Effluent	Influent	Effluent	(NmL)	(mmol/L)
B-500	500/500	386±5	431±30	32±8	27±7	90.7	5.51 (71%)
B-1000	1,500/1,000	999±22	1,011±25	40±9	41±5	153.3	8.95 (74%)
B-2000	2,500/2,000	1,444±120	1,361±135	44±9	141±28	231.9	11.8 (69%)
B-3000	-	4,937	4,287±133	66	336±22	-	-
FB-2-1000	1,500/1,000	978±18	962±40	30±3	36±6	-	8.21 (70%)
FB-4-1000	1,500/1,000	958±64	960±70	30±6	30±9	-	8.04 (71%)
FB-2-2000	3,000/2,000	1,866±37	1,831±42	35±9	85±17	-	11.4 (69%)
FB-4-2000	3,000/2,000	1,875±39	1,799±48	27±6	90±18	-	11.3 (70%)

CH_4 proportion in the CH_4/CO_2 mixture is shown enclosed in parentheses

B batch operation, *FB* fed-batch operation (2–2 h, 4–4 h), *BA* bicarbonate alkalinity

Table 3 Mean values of monitored variables.

Condition	TS (mg/L)		TVS (mg/L)		TSS (mg/L)		VSS(mg/L)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
B-500	871±32	848±83	532±63	522±135	48±8	38±7	44±7	26±10
B-1000	1,210±130	1,148±59	705±88	656±11	112±21	65±12	62±15	44±17
B-2000	2,874±108	2,661±251	1,245±81	1,104±108	110±23	108±18	92±18	89±16
B-3000	7,072	6,272	1,652	1,494	262	236	202	156
FB-2-1000	1,973±174	1,722±177	1,068±101	899±90	74±25	58±16	62±19	47±14
FB-4-1000	2,011±95	1,973±115	1,058±182	1,032±156	87±13	57±18	62±12	29±11
FB-2-2000	3,271±238	3,057±83	1,352±282	1,167±61	126±22	98±13	108±23	79±17
FB-4-2000	2,995±260	2,916±208	1,198±160	1,001±107	141±26	96±9	103±22	77±8

B batch operation, *FB* fed-batch operation (2–2 h, 4–4 h), *TSS* total suspended solids, *VSS* volatile suspended solids

The *second condition* was 45 days (135 cycles) and 10-min fill time, with non-filtered influent concentration of 1,060 mgCOD/L, resulting in an applied volumetric organic load of 1.29 gCOD/L.d, and influent supplementation of 1,500 $mgNaHCO_3/L$, after performing four stages to optimize this supplementation. System stability could be verified and removed volumetric organic load of 1.10 gCOD/L.d was obtained for 85% removal efficiency, in terms of filtered samples. The total volatile acid concentration was low, both in the influent

Table 4 Mean values of monitored variables.

Condition	AVOL (gCOD/L.d)	SAOL (mgCOD/gTVS.d)	$RVOL_{SF}$ (gCOD/L.d)	$SROL_{SF}$ (mgCOD/gTVS.d)	$Y_{V\text{-}CH4/COD}/Y_{C\text{-}CH4/COD}$ ($NmLCH_4$/gCOD)/ ($mmolCH_4$/gCOD)
B-500	0.60	15.2	0.57	14.5	95.5/11.8
B-1000	1.29	32.8	1.10	28.0	83.8/9.93
B-2000	2.44	62.1	1.78	45.3	78.1/8.10
B-3000	3.82	97.2	1.87	47.6	–/–
FB-2-1000	1.27	32.3	1.08	27.5	–/9.18
FB-4-1000	1.33	33.8	1.13	28.8	–/8.52
FB-2-2000	2.44	62.1	1.95	49.6	–/7.64
FB-4-2000	2.36	60.0	1.82	46.3	–/7.00

B batch operation, *FB* fed-batch operation (2–2 h; 4–4 h)

$C_{X\text{-}TS}$=45.2 gTS/L

$C_{X\text{-}TVS}$=39.3 gTVS/L and X_{TVS}=196.5 gTVS (considering 5 L of liquid medium in the reactor)

$\mathrm{AVOL} = \frac{(V_I \cdot n) \cdot C_I}{V}$

$\mathrm{SAOL} = \frac{(V_I \cdot n) \cdot C_I}{X_{TVS}}$

$\mathrm{RVOL_{SF}} = \frac{(C_I - C_{SF}) \cdot V_I}{V \cdot t_C}$

$\mathrm{SROL_{SF}} = \frac{(V_I \cdot n) \cdot (C_I - C_{SF})}{X_{TVS}}$

$Y_{V\text{-}CH4/COD} = (V_{CH4})/(CORA_{SF} \cdot V \cdot t_C)$

$Y_{C\text{-}CH4/COD} = (C_{CH4})\ (C_I \cdot \varepsilon_{SF})$

(40 mgHAc/L) and in the effluent (41 mgHAc/L), with generation of bicarbonate alkalinity (influent 999 mgCaCO$_3$/L and effluent 1011 mgCaCO$_3$/L). The volume of methane produced per cycle was 153.3 NmL for a concentration of 8.95 mmol/L and composition of 74% (assuming CH$_4$ and CO$_2$ only), i.e., for 5-L medium volume and 8-h cycle, the ratio between the volume of methane produced and the amount of organic matter removed was 83.8 NmLCH$_4$/gCOD, and the ratio between methane concentration and the difference of organic matter concentration (non-filtered influent and effluent) was 9.93 mmolCH$_4$/gCOD.

The *third condition* was 35 days (105 cycles) and 10-min fill time, with non-filtered influent concentration of 1,996 mgCOD/L, resulting in applied volumetric organic load of 2.44 gCOD/L.d, with influent supplementation of 2,500 mgNaHCO$_3$/L. System stability could be verified and removed volumetric organic load of 1.78 gCOD/L.d was obtained for 73% removal efficiency, in terms of filtered samples. Total volatile acid concentration was 141 mgHAc/L in the effluent, which exceeded that in the influent (44 mgHAc/L), with bicarbonate alkalinity consumption in the effluent (1,361 mgCaCO$_3$/L) in relation to the influent (1,444 mgCaCO$_3$/L). This shows that, although stability was attained, the system alkalinity reserve at the end of the cycle was lower than the alkalinity obtained from the influent supplementation and the alkalinity generated by the system, due to accumulation of volatile acids in the effluent, thus indicating that the cycle time was approaching the consumption limit in the methanogenesis stage of volatile acids, generated in the acidogenesis stage. The volume of methane produced per cycle was 231.9 NmL for a concentration of 11.8 mmol/L and composition of 69% (assuming CH$_4$ and CO$_2$ only), i.e., for 5-L medium volume and 8-h cycle, the ratio between the volume of methane produced and the amount of organic matter removed was 78.1 NmLCH$_4$/gCOD, and the ratio between methane concentration and the difference of organic matter concentration (non-filtered influent and effluent) was 8.10 mmolCH$_4$/gCOD.

The *fourth condition* was 10 days (30 cycles) and 10-min fill time, with influent concentration of 3,137 mgCOD/L, resulting in applied volumetric organic load of 3.82 gCOD/L.d). The operation was divided into three stages (stages I to III). In stage I alkalinity supplementation was 4,500 mgNaHCO$_3$/L, obtaining removal efficiency of 44% after 5 days (15 cycles) of operation and total volatile acid concentration of 56 mgHAc/L in the influent and 207 mgHAc/L in the effluent. It was thus decided to increase the alkalinity supplemented to the influent to 6,000 mgNaHCO$_3$/L in stage II, resulting in a removal efficiency of 47% and increase in total volatile acid concentration in the effluent to 270 mgHAc/L after 2 days (six cycles) of operation. Thus, in stage III alkalinity supplementation to the influent was 8,000 mgNaHCO$_3$/L, and after 3 days (nine cycles) removal efficiency did not increase and remained at 50%. Also, no reduction occurred in the total volatile acid concentration of the effluent, which remained at 336 mgHAc/L. Therefore, analysis of the ASBR behavior with influent concentration of 3,000 mgCOD/L showed that the strategy to increase alkalinity supplementation to the influent was not effective, i.e., the system did not reach operational stability. Organic matter removal efficiency was only 52% with high total volatile acid concentration (336 mgHAc/L) and high bicarbonate alkalinity consumption in the influent (4,937 mgCaCO$_3$/L) when compared to the effluent (4,287 mgCaCO$_3$/L). Hence, the objective to determine the operating limit of the ASBR under batch feeding operation was accomplished.

Effect of Fill Time in the Fed-Batch Feeding Followed by Batch

In the second phase (next four conditions), the influent concentration was kept at 1,000 and 2,000 mgCOD/L and the effect of fill time on system efficiency and stability was assessed, i.e., the purpose was to study feed strategy, by keeping the same volume of influent treated per cycle, but varying the fill time, changing batch feeding of 10-min fill time and fill time to total cycle time ratio of 0.02, to fed-batch feeding of 2- and 4-h fill time and fill time to total cycle time ratio of 0.25 and 0.50, respectively (Tables 1, 2, 3, and 4).

The *fifth condition* was 37 days (111 cycles) and 2-h fill time, with influent concentration of 1,052 mgCOD/L, resulting in applied volumetric organic load of 1.27 gCOD/L.d, with influent supplementation of 1,500 mg$NaHCO_3$/L. System stability could be verified and removed volumetric organic load of 1.08 gCOD/L.d was obtained for 85% removal efficiency, in terms of filtered samples. Total volatile acid concentration was 36 mgHAc/L in the effluent, which is slightly higher than that in the influent (30 mgHAc/L), maintaining approximately the same bicarbonate alkalinity of the effluent (962 mg$CaCO_3$/L) when compared to the influent (978 mg$CaCO_3$/L). The concentration of methane produced per cycle was 8.21 mmol/L and composition of 70% (assuming CH_4 and CO_2 only), i.e., the ratio between methane concentration and the difference of organic matter concentration (non-filtered influent and effluent) was 9.18 mmolCH_4/gCOD.

The *sixth condition* was 96 days (288 cycles) and 4-h fill time, with influent concentration of 1,110 mgCOD/L, resulting in applied volumetric organic load of 1.35 gCOD/L.d, with effluent supplementation of 1500 mg$NaHCO_3$/L. System stability could be verified and removed volumetric organic load of 1.13 gCOD/L.d was obtained for 85% removal efficiency, in terms of filtered samples. Total volatile acid concentration and bicarbonate alkalinity were 30 mgHAc/L and 959 mg$CaCO_3$/L, respectively, both in the effluent and influent, i.e., the values were equal, thus evidencing system stability. The concentration of methane produced per cycle was 8.04 mmol/L and composition of 71% (assuming CH_4 and CO_2 only), i.e., the ratio between methane concentration and the difference of organic matter concentration (non-filtered influent and effluent) was 8.52 mmolCH_4/gCOD.

Therefore, analysis of the effect of fill time for the condition with influent concentration of 1,000 mgCOD/L shows that the system virtually exhibited the same results of the batch feeding operation (fill time to total cycle time ratio of 0.02), i.e., feeding may be carried out in batch mode or fed-batch mode during 2 or 4 h (fill time to total cycle time ratio of 0.25 and 0.50, respectively), without impairing the final quality of the effluent and methane generation.

The *seventh condition* was 56 days (168 cycles) and 2-h fill time, with influent concentration of 1,866 mgCOD/L, resulting in applied volumetric organic load of 2.44 gCOD/L.d, with effluent supplementation of 3,000 mg$NaHCO_3$/L. System stability could be verified and removed volumetric organic load of 1.95 gCOD/L.d was obtained for 80% removal efficiency, in terms of filtered samples. Total volatile acid concentration was 85 mgHAc/L in the effluent, which is higher than that in the influent (35 mgHAc/L), maintaining approximately the same effluent bicarbonate alkalinity value (1,831 mg$CaCO_3$/L) when compared to the influent (1,866 mg$CaCO_3$/L). The concentration of methane produced per cycle was 11.4 mmol/L and composition of 69% (assuming CH_4 and CO_2 only), i.e., the ratio between methane concentration and the difference of organic matter concentration (non-filtered influent and effluent) was 7.64 mmolCH_4/gCOD.

The *eighth condition* was 37 days (111 cycles) and 4-h fill time, with influent concentration of 1875 mgCOD/L, resulting in applied volumetric organic load of 2.36 gCOD/L.d, with effluent supplementation of 3,000 mg$NaHCO_3$/L. System stability could be verified and removed volumetric organic load of 1.82 gCOD/L.d was obtained for 90% removal efficiency, in terms of filtered samples. Total volatile acid concentration was 90 mgHAc/L in the effluent, which is higher than that in the influent (27 mgHAc/L), maintaining approximately the effluent bicarbonate alkalinity (1,799 $mgCaCO_3/L$) when compared to the influent (1,875 $mgCaCO_3/L$). The concentration of methane produced per cycle was 11.3 mmol/L and composition of 70% (assuming CH_4 and CO_2 only), i.e., the ratio between methane concentration and the difference of organic matter concentration (non-filtered influent and effluent) was 7.00 $mmolCH_4/gCOD$.

Therefore, analysis of the effect of fill time for the condition with influent concentration of 2,000 mgCOD/L showed that the system virtually exhibited improvement in terms of removal efficiency and effluent quality when compared to the batch feeding operation (fill time to total cycle time ratio of 0.02), i.e., the 2- or 4-h fed-batch operation (fill time to total cycle time ratio of 0.25 and 0.50, respectively) improved the final quality of the effluent and maintained methane generation.

It should be mentioned that the methane production under fed-batch feeding followed by batch was not measured directly, but analyzed in terms of concentration, due to variation in the reactor volume caused by the gradual influent feeding along the cycle. Although this analysis, by using the concentration ratio, is prone to error because it depends on the biogas volume between the liquid medium surface and the reactor cover, where the biogas sample was collected, it is possible to estimate the volume generated by comparing with the batch feeding conditions, in which both values were measured.

Profiles Along the Operation Cycle

The first-order kinetic model was initially fitted to the experimental values for operations with influent concentration from 500 to 2,000 mgCOD/L and 10-min fill time (batch mode). In this model, the existence of residual concentration was considered both for organic matter (C_{SR}) and total volatile acids (C_{TVAR}).

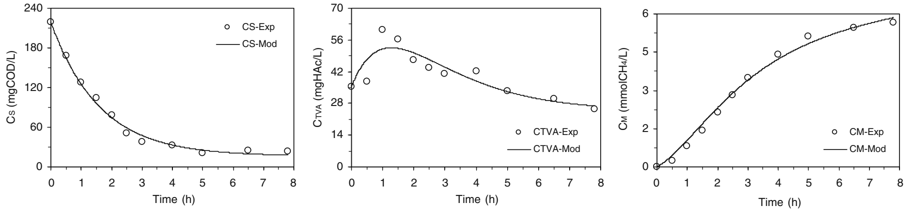
Table 5 contains the values of C_{SR}, C_{TVAR}, and kinetic parameters (k_{1S}, k_{1TVA}, k_{2TVA}, and k_{2M}), in addition to the respective value of the correlation coefficient square (R^2). Figure 2 contains the results of organic matter concentration profiles (C_S), total volatile acid concentration (C_{TVA}), and methane concentration (C_M), by comparing experimental points and fitted models.

Comparison of the values of the first-order kinetic parameters and residual organic matter concentrations, obtained by fitting the model, shows that the increase in the influent concentration from 500 to 1,000 mgCOD/L resulted in reduction in the kinetic parameter for organic matter consumption (k_{1S}), but the residual organic matter concentration

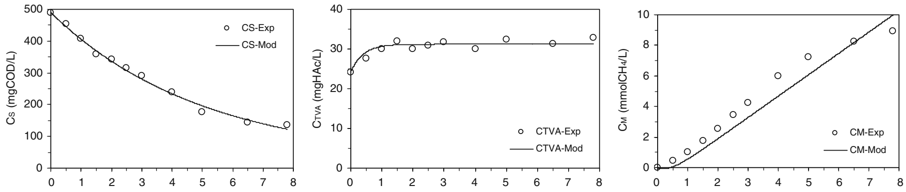
Table 5 Summary of the kinetic fit for batch and fed-batch operations.

Condition	k_{1S} (h^{-1})	k_{1TVA} (h^{-1})	k_{2TVA} (h^{-1})	k_{2M} (h^{-1})	C_{SRA} (mgCOD/L)	C_{TVAR} (mgHAc/L)	R^2
B-500	0.62	0.21	0.68	0.040	16.3	24.8	0.961
B-1000	0.20	0.01	2.5	0.326	24.3	31.2	0.963
B-2000	0.24	0.02	0.01	0.020	449.5	62.0	0.978
FB-2-1000	0.18	0.02	0.13	0.07	47.2	0.00	0.867
FB-4-1000	0.50	0.03	0.14	0.10	108.0	0.00	0.932
FB-2-2000	0.47	0.01	0.00	0.05	467.4	30.4	0.936
FB-4-2000	0.70	0.02	0.00	0.09	423.4	65.8	0.901

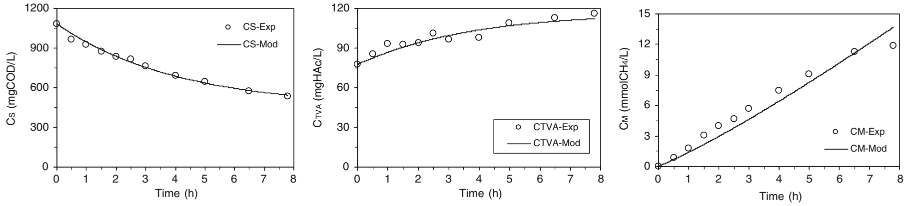
B batch operation, *FB* fed-batch operation (2–2 h; 4–4 h)



500 mgCOD/L



1000 mgCOD/L



2000 mgCOD/L

Fig. 2 C_S, C_{TVA}, and C_M profiles in batch operation

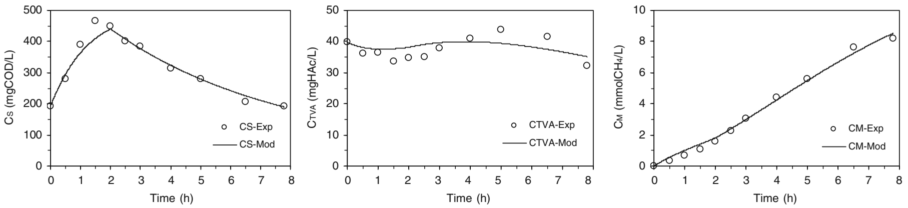
remained approximately constant (C_{SR}), indicating continuing reduction in the residual organic matter concentration in the 1,000 mgCOD/L assay. It can thus be assumed that there is a potential need to increase cycle length to achieve higher organic matter removal.

An increase in the influent concentration from 1,000 to 2,000 mgCOD/L did not have any significant effect on the variation of the kinetic parameter of organic matter consumption, but there was an increase in the residual organic matter concentration, indicating that at this condition the potential need for increase in cycle length still exists; however, this would promote a less significant increase in organic matter removal when compared to the previous condition, i.e., the system would reach the operational limit of stability and efficiency.

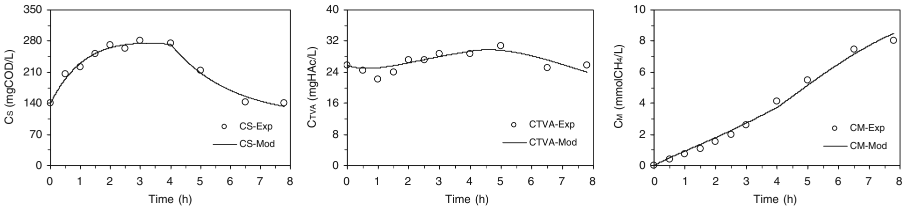
The operation condition with influent concentration of 500 mgCOD/L was that which presented a higher value of the kinetic parameter and lower residual organic matter concentration, indicating this condition was the best for the anaerobic process, probably due to lowest accumulation levels of intermediate compounds of the anaerobic metabolism (volatile acids) and of the compounds present in this residue of the biodiesel production process (unreacted methanol and soy oil and non-separated biodiesel).

Regarding the kinetic parameter for organic matter consumption obtained by fitting the experimental values for total volatile acids (k_{1TVA}), the value of k_{1TVA} was impaired in the 1,000 mgCOD/L assay by the presence of approximately constant values, showing mathematical limitation of this model, despite the fact that the profiles in Fig. 2 showed that the increase in influent concentration was leading to volatile acids accumulation, thus indicating approach of the operational limit of the reactor. Regarding the kinetic parameter of total volatile acids consumption obtained by fitting the experimental values for total volatile acids (k_{2TVA}), the same comment may be used in terms of fitting limitations due to the presence of approximately constant values. However, for values obtained at the 500 and 2,000 mgCOD/L conditions there is a direct relation between the values of k_{2TVA} parameters and the tendency of volatile acids accumulation, which would indicate the approach of the operational limit of the reactor. Regarding the kinetic parameter of total volatile acids consumption obtained by fitting the experimental values of total volatile acids and methane (k_{2M}), the same comment may be used in terms of fitting limitations due to the presence of approximately constant values.

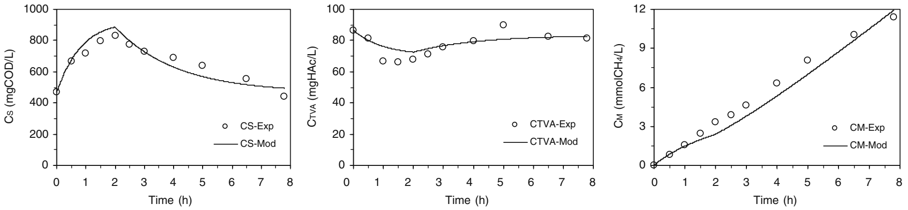
The first-order kinetic model was also used to fit the experimental values for the 1,000 and 2,000 mgCOD/L conditions with 2- and 4-h fed-batch feeding. Table 5 contains the values of C_{SR}, C_{TVAR}, and kinetic parameters (k_{1S}, k_{1TVA}, k_{2TVA}, and k_{2M}), in addition to the respective values of the correlation coefficient square (R^2). Figure 3 contains the results of the organic matter concentration (C_S), total volatile acid concentration (C_{TVA}), and methane concentration (C_M) profiles, comparing experimental points and fitted models.



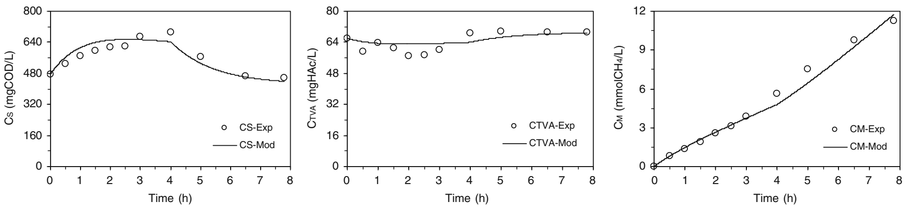
1000 mgCOD/L and 2-h fill time



1000 mgCOD/L and 4-h fill time



2000 mgCOD/L and 2-h fill time



2000 mgCOD/L and 4-h fill time

Fig. 3 C_S, C_{TVA}, and C_M profiles in fed-batch operation

Comparison of the 1,000 mgCOD/L condition with batch and fed-batch feeding (Table 5 and Figs. 2 and 3) shows that the kinetic parameter k_{1S} (which quantifies the organic matter consumption and is fitted by the organic matter data) was virtually the same for 2-h fed-batch operation and increased for the 4-h fed-batch operation. It can thus be concluded that, although the efficiency in the end-of-cycle condition has been the same for the three fill times, the 4-h fed-batch operation reached this final stability level in a shorter time interval, evidencing that gradual feeding may provide the advantage of a possible shorter cycle length, as well as a reduction in the maximum concentration value of organic matter achieved along the cycle in the reactor. Yet again, analysis of the kinetic parameters obtained by the volatile acid concentration profiles was impaired due to the behavior of the values achieved, which did not exhibit a defined trend because of the approximately constant values.

Comparison of the 2,000 mgCOD/L condition with batch and fed-batch feeding (Table 5 and Figs. 2 and 3) shows that the kinetic parameter k_{1S} (which quantifies the organic matter consumption and is fitted by the organic matter data) was higher for 2- and 4-h fed-batch feeding. It can thus be concluded that higher fill times favor organic matter consumption at higher influent concentrations, i.e., under conditions where the accumulation of intermediate products from the anaerobic digestion is higher. Therefore, in this case, in addition to obtaining lower concentration at the end-of-cycle condition, the maximum value of organic matter concentration, obtained along the cycle inside the reactor, also decreased. Again, analysis of the kinetic parameters obtained from the volatile acid concentration profiles was impaired due to the behavior of the values achieved, which did not exhibit a defined trend because of the approximately constant values.

Conclusions

In batchwise mode the organic matter removal were 93%, 81%, and 66% for influent of 500, 1,000, and 2,000 mgCOD/L and alkalinity of 500, 1,500, and 2,500 mgNaHCO$_3$. In fed-batchwise mode the organic matter removal was 85% at 1,000 mgCOD/L and 2- and 4-h fill times, and 80% and 77% at 2,000 mgCOD/L and 2- and 4-h fill times. Gradual feeding improved removal and smoothed the organic matter and volatile acids along the cycle. The ratio of formed methane and organic matter consumption was 96 NmLCH$_4$/gCOD. A first-order kinetic model distinguishes between efficiencies in the end-of-cycle and along the cycle.

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