



AN SBBR APPLIED TO BIOMETHANE PRODUCTION FOR VINASSE TREATMENT: EFFECTS OF ORGANIC LOADING, FEED STRATEGY AND TEMPERATURE

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Abstract – This study assessed the feasibility of an anaerobic sequencing batch and fed-batch biofilm reactor (AnSBBR) with mechanical stirring for vinasse treatment and biomethane production. The amount of liquid medium used was 3 L, and the treated volume was 1 L per cycle. The cycle time was 8 h, with feeding times of 10 min (batch mode) and 240 min (fed-batch mode). Methane productivity increased with the increase in organic load from 1.0 to 10.0 gCOD.L⁻¹.d⁻¹. The maximum molar productivity and yield of methane were 123.4 molCH₄.m⁻³.d⁻¹ and 13.8 mmolCH₄.gCOD⁻¹ (88% of the theoretical), respectively. These parameters were lower in fed-batch mode than in batch mode and were lower at 45°C than at 30°C due to biomass adaptation. In general, the values of the kinetic parameters increased with organic load and were similar for the batch and fed-batch modes. Therefore, the increase in organic load positively influenced the process by increased COD removal and lower volatile acids accumulation as the load increased. The batch mode performed better than did the fed-batch mode and 30°C was more suitable than 45°C to produce methane under the studied conditions.

Keywords: AnSBBR; biomethane; applied organic load; feed strategy; temperature.

INTRODUCTION

Vinasse disposal in soil is widely used because vinasse is rich in many nutrients and minerals that are essential to plant growth. However, fertirrigation with vinasse may change physical and chemical characteristics of the soil. This activity leads to the soil salinisation, mainly due to the high concentration of potassium in vinasse. These issues show that there is a need to develop more suitable use options for this type of ethanol production effluent (Santos et al., 2013). Biological treatment is recognized as

an effective method of treatment for the highly polluted wastewaters that result from agroindustry, including distilleries. Anaerobic treatment can be used to convert a significant portion of the organic matter into biogas, which can be used as an energy source in the distillery (Pant and Adholeya, 2007; Vlissidis and Zouboulis, 1993; Wilkie et al., 2000; Cesaro and Belgioro, 2015).

Within this framework, the anaerobic sequencing batch reactors, ASBR (free cells) and AnSBBR (immobilized cells), are being largely studied for bioenergy generation and environmental compliance (Zaiat et al., 2001). Arooj et al. (2008) and Kim et al. (2005) used an ASBR for

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hydrogen production and they analysed the effects of the hydraulic retention time on the process and the effects of the substrate concentration, respectively.

Other researchers used an ASBR for methane production: Massé et al. (2004), studying the effect of scale up; Cassidy et al. (2008a; 2008b), assessing the influence of the organic load rate, solid retention time, and food/microorganism rate; Selma et al. (2010), analysing the effects of organic load (by increasing influent concentration) and the feed strategy (batch or fed-batch); and Silva et al. (2013), varying the organic load, the reactor feed time and the cycle length. On the other hand, Ndegwa et al. (2005), aiming at reactor optimization, and Ruiz et al. (2002), investigating the kinetics of the degradation process, used ASBR for biogas production.

Finally, some papers related to AnSBBR and methane production regarding feed strategy and organic load improvement: Lovato et al. (2012) studied the effects of feed strategy treating the effluent from biodiesel production; Bezerra et al. (2011) treated the same effluent to test the effect of the organic load; and Lullio et al. (2014) aimed at integrating hydrogen and methane production treating the effluent of hydrogen production to produce methane. Only a few studies involved the use of an AnSBBR to produce methane, and there are no papers in the published literature regarding the use of vinasse as a substrate. In addition, note that the anaerobic process is assuming importance in bioenergy production (hydrogen and methane) via the biogas resulting from the biotransformation of polluting substances.

In view of the aspects presented, this study analysed the performance of an anaerobic sequencing batch and fed-batch biofilm reactor (AnSBBR) with mechanical stirring for vinasse treatment and biomethane production. The influence of the applied volumetric organic load (AVOL – from 1.0 to 10.0 gCOD.L⁻¹.d⁻¹), feed strategy (feeding time of 10 min in batch mode and 240 min in fed-batch mode) and temperature (30°C and 45°C) on the yield of methane from the consumed organic load, methane productivity, kinetic parameters and the overall factors of the degradation process were assessed.

MATERIALS AND METHODS

AnSBBR with immobilised biomass and mechanical stirring

Figure 1 presents the scheme of the bioreactor with mechanical stirring (New Brunswick Scientific model Bioflo 110[®]), which was composed of a glass vessel of 20 cm in diameter and 30 cm in height, with a total volume of 7.5 L and a useful capacity of 6.0 L. The support material was confined in a pierced stainless steel-316 container of 18 cm in height and 7.0 cm and 17.5 cm of internal and external diameter, respectively. Fixed stirring at 300 rpm

was implemented via a motor connected to the turbine type impellers of 6 cm in diameter, composed of two sets of six plane blades (Rushton pattern) installed at 8 and 16 cm from the bottom of the vessel (Michelan et al., 2009).

Feeding and discharge were performed using diaphragm pumps (Prominent models β and Concept, respectively). An automation system composed of temporizers was responsible for starting/stopping the pumps and for stirring to implement the steps of sequencing batch operation: feeding, reaction and discharge. The temperature was controlled by water circulation into the double external wall of the reactor (Marconi model MA-184).

Inoculum and inert support

The inoculum was obtained from an UASB that treated wastewater of a slaughterhouse of poultry. The inoculum presented values of total solid concentration (TS) and total volatile solids (TVS) of 62 and 51 g.L⁻¹, respectively. The material used as support was polyurethane foam (cubes of 1.0 cm), with an apparent density of 23 kg.m⁻³ and a porosity of approximately 95%. The anaerobic sludge was immobilized according to Zaiat et al. (1994). The support was confined inside the reactor (pierced stainless steel-316 container with 7 cm from the center) so that the impeller could freely rotate (Figure 1).

Wastewater

The wastewater/substrate was based on the vinasse that results from the industrial process of bioethanol production from a sugar/alcohol plant located in São Paulo, Brazil, which is produced during the purification of ethanol by distillation from the medium fermented in the previous stage. The composition of the industrial vinasse used is presented in Table 1.

According to the experimental condition, the industrial vinasse was diluted in tap water and supplemented with urea (CH₄N₂O – 5.8 mg.L⁻¹ for each 1000 mgCOD.L⁻¹) and sodium bicarbonate (NaHCO₃ – 500 mg.L⁻¹ for each 1000 mgCOD.L⁻¹)

Physical-chemical analyses and microbiological tests

The following parameters were monitored in both the influent and the effluent: organic matter concentration non-filtered (C_{ST}) and filtered (C_{SP}) (as chemical oxygen demand), bicarbonate alkalinity (BA), total volatile acids (TVA), total solids (TS), total volatile solids (TVS), total suspended solids (TSS) and volatile suspended solids (VSS), as well as the pH (*Standard Methods for the Examination of Water and Wastewater*, 1995).

The analysis of biogas composition was performed by gas chromatography using an Agilent[®] model 7890 chromatograph that was equipped with a thermal

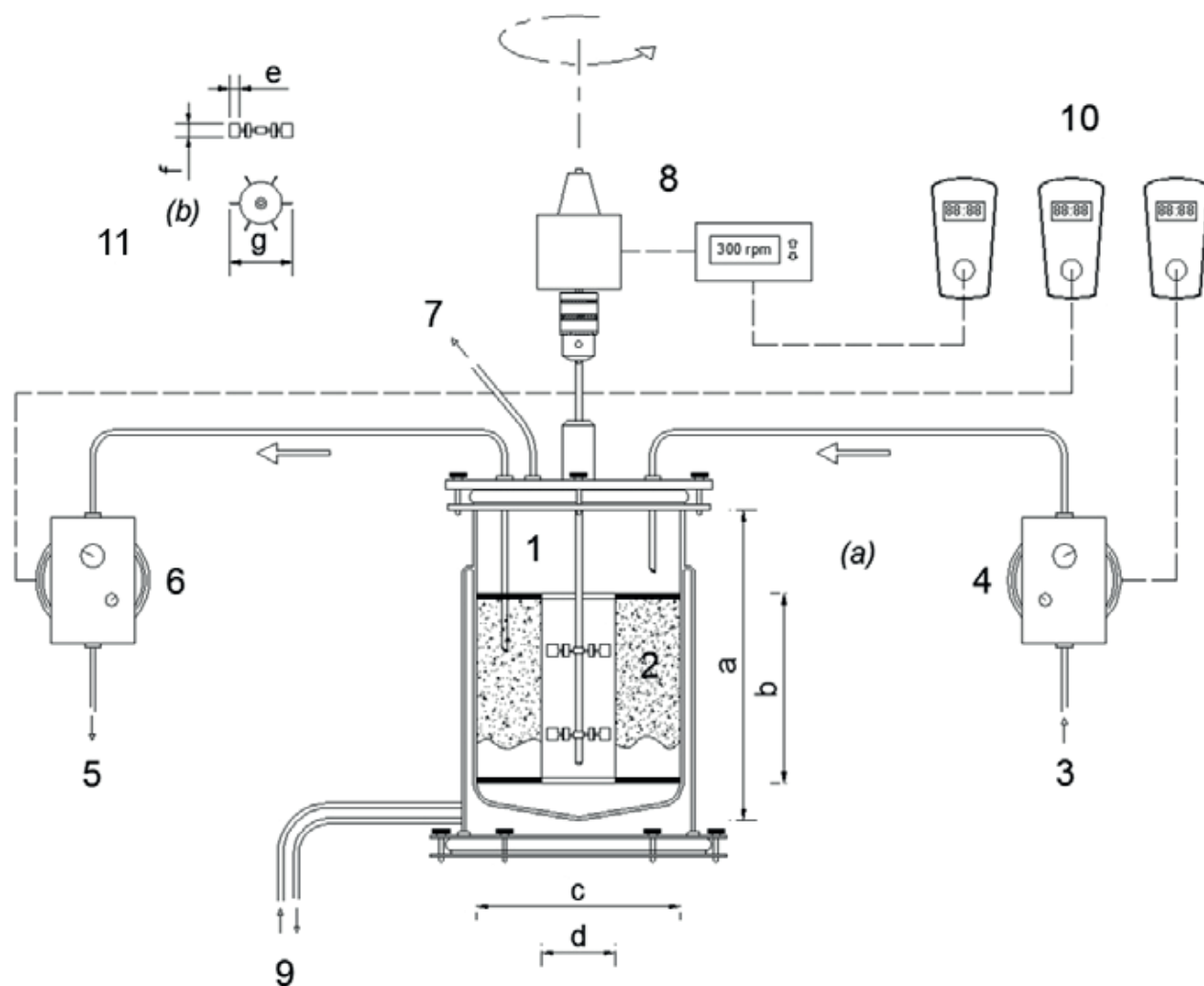


Figure 1. Scheme of the AnSBBR: (1) Bioreactor (a = 29,5 cm; b = 18 cm; c = 18 cm); (2) Stainless steel box (b = 18 cm; d = 7 cm); (3) Influent; (4) Feed pump; (5) Effluent; (6) Discharge pump; (7) Biogas outlet; (8) Stirring; (9) Thermostatic bath; (10) Automation system; (11) Turbine type propellers with six plane blades (e = 2 cm; f = 1.5 cm; g = 6 cm).

Table 1. Composition of the industrial vinasse.

Parameter	Concentration (mg.L ⁻¹) *
COD	31258
pH	5.6
BA	27
TVA	7633
TVS	19573
TSS	1524
VSS	1182
FSS	341
VDS	18391
EtOH	248
HAc	1339
HPr	91
HBu	0 **
HVa	0 **
HCa	0 **

* Standard deviation = 15% (5 samples). ** < Limit of detection estimated as 3 mg.L⁻¹.

conductivity detector and GS-Carbonplot column with $30\text{ m} \times 0.53\text{ mm} \times 3.0\text{ }\mu\text{m}$ of film thickness. The carried gas was argon, with a flow of $3.67\text{ mL}\cdot\text{min}^{-1}$, an injector temperature of 185°C , a *split* ratio of 10 and an injection volume of $200\text{ }\mu\text{L}$. The temperature of the oven was programmed as 40°C isothermal in 5 min. The temperature of the detector was 150°C , with a flow *make up* of argon of $8.33\text{ mL}\cdot\text{min}^{-1}$. The total biogas production during a cycle (V_G) was measured using the Ritter gas measurer model MilligasCounter.

The analysis of anaerobic metabolism (acetone, methanol, ethanol, n-butanol, acids acetic, propionic, butyric, isobutyric, valeric, isovaleric and caproic) was performed via gas chromatography (method of *head-space*) with external standard (isobutanol and crotonic acid) using an Agilent® model 7890 chromatograph equipped with a flame ionization detector and a HP-Innowax column with dimensions of $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ of film thickness. The carried gas was hydrogen, with a flow of $1.56\text{ mL}\cdot\text{min}^{-1}$, an injector temperature of 250°C , a *split* ratio of 10 (*head-space*) and an injection volume of $400\text{ }\mu\text{L}$, using an automatic injector. The temperature of the oven was programmed as follows: from 35°C to 38°C at $2^\circ\text{C}\cdot\text{min}^{-1}$, from 38°C to 75°C at $10^\circ\text{C}\cdot\text{min}^{-1}$, from 75°C to 120°C at $35^\circ\text{C}\cdot\text{min}^{-1}$, at 120°C for 1 min, from 120°C to 170°C at $10^\circ\text{C}\cdot\text{min}^{-1}$ and at 170°C for 2 min (*head-space*). The temperature of the detector was 280°C (*head-space*), with a hydrogen flux of $30\text{ mL}\cdot\text{min}^{-1}$, a synthetic air flow of $300\text{ mL}\cdot\text{min}^{-1}$ and a flow *make up* of nitrogen of $30\text{ mL}\cdot\text{min}^{-1}$.

Samples from the reactor were collected for microbiological examination. The samples were examined on glass slides using an Olympus model BX41® optical microscope with Optronics digital camera system and the Image-Pro Plus® Version 4.5.0 image acquisition software.

Experimental procedure

The experimental conditions were produced using different volumetric organic loads that were modified by the influent concentration, which ranged from $1000\text{ mgCOD}\cdot\text{L}^{-1}$ to $10000\text{ mgCOD}\cdot\text{L}^{-1}$. Experiments were performed in batch mode at 30°C that used vinasse as the influent (Conditions C01 to C08 – from $1000\text{ mgCOD}\cdot\text{L}^{-1}$ to $10000\text{ mgCOD}\cdot\text{L}^{-1}$). In sequence, the feeding strategy was changed from batch to fed-batch mode while maintaining the influent concentration of vinasse at $10000\text{ mgCOD}\cdot\text{L}^{-1}$ and the temperature at 30°C (Condition C09). The last condition was performed in batch mode with an influent concentration of $10000\text{ mgCOD}\cdot\text{L}^{-1}$, with the temperature increased from 30°C (from the other conditions) to 45°C , while maintaining the operation in batch mode (Condition C10). In all experimental conditions, the cycle time length was 8 hours, and the stirring was 300 rpm (Bezerra et al., 2011; Lovato et al., 2012; Selma et al., 2010; Silva et al., 2013).

The reactor operation proceeded as follows: (a) feed of 1.0 L of wastewater in 10 min (batch mode) or 240 min (fed-batch mode); (b) agitation and reaction (for fed-batch mode, the feeding time is included); and (c) discharge of 1.0 L in 10 min. A cycle was completed while maintaining a residual volume of 2.0 L from the total volume of liquid medium in the reactor of 3.0 L. In sequence, a new cycle started.

Once stable experimental conditions were achieved, profiles along the cycle for some of the monitored parameters were made. The parameters of interest were filtered organic matter concentration, bicarbonate alkalinity, total volatile acids, intermediate metabolites, biogas (volume and composition) and pH. The total volume sampled had a maximum of 300 mL, which is 10% of the system volume. Thus, after the profiles were performed a new experimental condition was implemented.

Theoretical

The efficiencies of total organic matter removal (ϵ_{ST}) are calculated using Equation (1), and the efficiencies of filtered organic matter removal (ϵ_{SF}) are calculated using Equation (2). The efficiencies of filtered organic matter removal (ϵ) for the profiles along the cycle are calculated using Equation (3). C_{SINF} and C_{SINFO} is the filtered organic matter concentration in the influent and in the reactor at the beginning of the cycle (time zero – the organic matter concentration in the reactor after the initial dilution of the influent), respectively.

$$\epsilon_{\text{ST}} (\%) = \frac{C_{\text{SINF}} - C_{\text{ST}}}{C_{\text{SINF}}} \times 100 \quad (1)$$

$$\epsilon_{\text{SF}} (\%) = \frac{C_{\text{SINF}} - C_{\text{SF}}}{C_{\text{SINF}}} \cdot 100 \quad (2)$$

$$\epsilon (\%) = \frac{C_{\text{SINFO}} - C_{\text{SF}}}{C_{\text{SINFO}}} \cdot 100 \quad (3)$$

The applied volumetric organic load (AVOL) is defined as the amount of organic matter that is applied to the reactor per unit time and per volume of medium ($\text{gCOD}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$). For reactors operated in batch mode, AVOL is calculated using Equation (4). The specific applied organic load (ASOL) is defined as the amount of organic matter that is applied to the reactor per unit time and per mass of total volatile solids in the reactor ($\text{gCOD}\cdot\text{gTVS}^{-1}\cdot\text{d}^{-1}$). For reactors operated in batch mode, ASOL is calculated using Equation (5).

The removed volumetric organic load (RVOL_F) for filtered samples is defined as the amount of organic matter

removed by the reactor per unit time and per volume of medium in the reactor (gCOD.L⁻¹.d⁻¹). For reactors operated in batch mode, RVOL_F is calculated using Equation (6). The specific removed organic load (RSOL_F) for filtered samples is defined as the amount of organic matter removed per unit time and per mass of total volatile solids in the reactor (gCOD.gTVS⁻¹.d⁻¹). For reactors operated in batch mode, RSOL_F is calculated using Equation (7).

$$AVOL = \frac{(V_F \cdot N) \cdot C_{SINF}}{V_R} \quad (4)$$

$$ASOL = \frac{(V_F \cdot N) \cdot C_{SINF}}{M_{TVS}} \quad (5)$$

$$RVOL_F = \frac{(V_F \cdot N) \cdot (C_{SINF} - C_{SF})}{V_R} \quad (6)$$

$$RSOL_F = \frac{(V_F \cdot N) \cdot (C_{SINF} - C_{SF})}{M_{TVS}} \quad (7)$$

Productivity (volumetric, VPr, or molar, MPr) is defined by the amount of methane produced per unit time and per usable reactor volume (mL-STP-CH₄.L⁻³.d⁻¹ or molCH₄.L⁻³.d⁻¹), as calculated using Equations (8) and (9). The specific productivity (volumetric, SVPr, or molar, SMPr) is defined as the amount of methane produced per unit time and per mass of total volatile solids in the reactor (mL-STP-CH₄.kgTVS⁻¹.d⁻¹ or molCH₄.kgTVS⁻¹.d⁻¹) and is calculated from Equations (10) and (11).

The volumetric yield of methane produced (mL-STP) per consumed load (gCOD) is defined as the amount of methane produced (in moles) by the amount of organic matter consumed (in mass). The molar volumetric yield of methane can be obtained by replacing the volume of produced methane (mL-STP) in Equation (12) with the amount of methane produced in moles, as found by using Equation (13).

$$VPr = \frac{N \cdot V_{CH4-STP}}{V_R} \quad (8)$$

$$MPr = \frac{N \cdot N_{CH4}}{V_R} \quad (9)$$

$$SVPr = \frac{N \cdot V_{CH4-STP}}{M_{TVS}} \quad (10)$$

$$SMPr = \frac{N \cdot N_{CH4}}{M_{TVS}} \quad (11)$$

$$Y_{V-CH4/COD} = \frac{V_{CH4-STP}}{V_F \cdot (C_{SAFF} - C_{SF})} \quad (12)$$

$$Y_{M-CH4/COD} = \frac{N_{CH4}}{V_F \cdot (C_{SAFF} - C_{SF})} \quad (13)$$

The first-order kinetic model applied was developed by Rodrigues *et al.* (2004), based on the model of Bagley and Brodtkorb (1999). The latter is an adaptation of another model that was proposed by the *International Water Association* (IWA) and was applied to systems of treatment with activated sludge. In the applied model, which was developed to sequence anaerobic batch reactors, the organic matter degradation process is simplified and divided into two consecutive steps. The first step is when the substrate is converted into total volatile acids, and in the second step, these acids are converted into methane. The model admits the existence of a residual concentration of both substrate and total volatile acids, in which the respective reaction rate tends to zero.

The organic matter degradation pathway is shown as follows, in which a, b and c are stoichiometric coefficients and k₁ and k₂ are the apparent kinetic parameters (Equation 14).



$$r_S = k_{1,S} \cdot (C_S - C_{SR}) \quad (15)$$

$$r_{TVA} = k_{1,TVA} \cdot (C_S - C_{SR}) - k_{2,TVA} \cdot (C_{TVA} - C_{TVAR}) \quad (16)$$

$$r_M = k_{2,M} \cdot (C_{TVA} - C_{TVAR}) \quad (17)$$

The equations of the reaction rate of substrate consumption (r_S), the formation of total volatile acids (r_{TVA}) and the formation of methane (r_M) are shown in Equations (15) to (17), with k_{1S}, k_{1TVA}, k_{2TVA} and k_{2M} being the same apparent kinetic parameters, but associated with substrate consumption, total volatile acids formation and methane formation, respectively. C_S and C_{TVA} are the substrate concentrations and the total volatile acids, and C_{SR} and C_{TVAR} are their respective residual values.

The mass balance of the reactor is shown in Equations (18) to (21). Two distinct conditions of operation must be considered: the first is in fed-batch mode, in which the feeding time is not negligible compared with the total

cycle length (i.e., $F \neq 0$), and the second is in batch mode, in which the feeding time is negligible (i.e., $F = 0$). The values of C_{S0} , C_{TVA0} , C_{M0} and V_0 correspond to the initial values of C_S , C_{TVA} , C_M and V , respectively. The values of C_{Sf} and C_{TVAf} correspond to the values of feeding of C_S and C_{TVA} .

$$\frac{dV}{dt} = F \quad t = 0 \rightarrow V = V_0 \quad (18)$$

$$\frac{dC_S}{dt} = \frac{F}{V} \cdot (C_{Sf} - C_S) - r_S \quad t = 0 \rightarrow C_S = C_{S0} \quad (19)$$

$$\frac{dC_{TVA}}{dt} = \frac{F}{V} \cdot (C_{TVAf} - C_{TVA}) + r_{TVA} \quad (20)$$

$$t = 0 \rightarrow C_{TVA} = C_{TVA0}$$

$$\frac{dC_M}{dt} = -\frac{F}{V} \cdot C_M + r_M \quad (21)$$

$$t = 0 \rightarrow C_M = C_{M0}$$

Because these are differential equations, Euler's numerical integration method and a constant integration step were applied and programmed in the software Excel® (Excel) to determine these parameters via Newton's algorithm using the Solver tool in Excel.

The non-specific kinetic parameters k_{IS} , k_{ITVA} , k_{2TVA} e k_{2M} are apparent because they encompass effects of mass transport and heat transfer. Specific kinetic parameters are the "non-specific" parameters divided by the biomass concentration (C_X – expressed by the total volatile solids in the reactor, TVS, in the volume of reacting medium) and are represented by k'_{IS} , k'_{ITVA} , k'_{2TVA} and k'_{2M} . The parameters C_{SR} and C_{TVAR} correspond only to an estimate of the residual value of the substrate and the volatile acids in the reactor at the end of a cycle. In addition, the kinetic model applied is a simplification of the complex process of anaerobic degradation.

RESULTS AND DISCUSSION

Analysis of the parameters monitored in the experimental conditions in batch mode (30 and 45°C) and fed-batch mode (30°C)

Table 2 presents the average values of the parameters monitored during all of the experimental conditions. Both the organic matter concentration (COD) and efficiency of removal during the increase in organic load in Conditions C01 to C08 (batch – 30°C) showed a significant

consumption of organic matter, with the efficiency of removal for filtered samples always greater than 90%.

For Conditions C09 (fed-batch – 30°C) and C10 (batch – 45°C), the organic matter concentration (COD) in the effluent increased, with efficiencies of removal for the filtered samples of 88% and 46%, respectively. The average pH values in all conditions (C01 to C10) were in the neutral range (7.2-7.7). The total volatile acids in the influent were consumed during the increase of the organic load (Conditions C01 to C08) and increased with the change in feed strategy and temperature (Conditions C09 and C10).

The increase in the organic load also led to a gradual increase in the volumes of biogas per cycle (Conditions C01 to C08). This result can be explained by the increase in the organic matter (COD) fed because under these conditions a greater amount of substrate that could be converted into biogas was fed to the reactor. In addition, favourable environmental conditions existed for the stability of the process. The maximum, the mean and minimum volumes of CH_4 produced were 2767, 1169 and 107 mL-STP- CH_4 ·Cycle⁻¹, respectively. However, there were decreases in the volumes of biogas that were produced per cycle with changes in the feed strategy and temperature (conditions C09 and C10), which resulted in volumes of 2297 and 785 mL-STP- CH_4 ·Cycle⁻¹, respectively.

Table 3 presents the performance indicators for the increase in organic load (conditions C01 to C08), the change in feed strategy from batch mode to fed-batch mode and the increase in temperature from 30°C to 45°C (conditions C09 and C10). Shading indicates the proportion of the magnitudes of parameters in the same line. Y_E/Y_T is the ratio of the experimental yield, both molar (M) and volumetric (V), to the theoretical yield of 15.63 mmol CH_4 ·gCOD⁻¹ or 350 mL CH_4 -STP·gCOD⁻¹. SMPr and SVPr are the specific molar and volumetric productivity indicators, respectively, and X_{CH_4} is the percentage of methane in biogas.

For the conditions in batch mode at 30°C (C01 to C08), Table 3 shows that there is a general tendency for the indicators to increase with an increase in organic load, and Condition C07 presented the best results. The yield indicator $Y_{M-CH_4/COD}$ shows that, overall, a greater number of CH_4 molecules were produced from the same quantity of consumed organic matter, i.e., the system became more efficient by increasing the organic load. Figure 2 shows a tendency to reach a maximum; as a result, using the fitted equation shown, the maximum yield was 13.7 mmol CH_4 ·gCOD⁻¹, corresponding to an AVOL of 7.50 gCOD·L⁻¹·d⁻¹ (batch at 30°C). This yield corresponds to 88% of the theoretical yield. In addition, the methane productivity increased with the increase in organic load. The maximum molar (MPr) reached was 123.4 mol CH_4 ·m⁻³·d⁻¹, and the volumetric (VPr) of 2767 mL-STP- CH_4 ·L⁻¹·d⁻¹. Figure 2 shows a tendency of increasing productivity towards this maximum in an AVOL of 10.1 gCOD·L⁻¹·d⁻¹.

Table 2. Experimental protocol and parameters monitored in the effluent and the organic loads for all experimental conditions.

Parameter	Condition	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10
T	(°C)	30	30	30	30	30	30	30	30	30	45
C _{INF}	(mgCOD.L ⁻¹)	1000	2000	3000	4000	5000	6000	8000	10000	10000	10000
Feed strategy		B	B	B	B	B	B	B	B	FB	B
C _{ST}	(mgCOD.L ⁻¹)	125	219	314	351	167	191	254	261	1150	5204
C _{SF}	(mgCOD.L ⁻¹)	109	167	286	384	129	154	224	235	1025	5036
ε _{ST}	(%)	89	89	90	92	97	97	97	97	88	46
ε _{SF}	(%)	90	92	91	91	98	98	97	98	90	48
pH	u	7.20	7.44	7.52	7.70	7.43	7.62	7.69	7.72	7.85	7.64
TVA	(mgHAc.L ⁻¹)	25	33	63	57	38	66	82	73	616	2591
BA	(mgCaCO ₃ .L ⁻¹)	492	967	1383	1819	1683	1982	2511	3383	4055	2367
TS	(mg.L ⁻¹)	862	1807	2365	2793	2361	2994	4138	4958	7532	9809
TDS	(mg.L ⁻¹)	832	1749	2317	2739	2323	2958	4100	4897	7331	9641
DVS	(mg.L ⁻¹)	347	583	541	433	426	630	969	1150	1693	3271
M _{TVS}	(g)	41.5	41.2	44.4	58.6	42.5	54.9	50.4	64.7	82.1	61.3
C _X	(g.L ⁻¹)	13.8	13.7	14.8	19.5	14.2	18.3	16.8	21.6	27.4	20.4
C _X '	(g.support ⁻¹)	0.76	0.82	0.88	0.95	0.80	1.21	1.16	1.72	1.86	1.25
V _{CH₄}	(mLCH ₄ .cycle ⁻¹)	107	352	627	911	1424	1815	2403	2767	2297	785
AVOL	(gCOD.L ⁻¹ .d ⁻¹)	1.09	2.09	3.01	4.12	5.16	6.19	8.18	10.07	9.67	10.31
RVOL	(gCOD.L ⁻¹ .d ⁻¹)	0.98	1.92	2.72	3.74	5.03	6.04	7.96	9.83	8.67	4.90
ASOL	(gCOD.gSVT ⁻¹ .d ⁻¹)	0.08	0.15	0.20	0.21	0.36	0.34	0.49	0.47	0.35	0.50
RSOL	(gCOD.gSVT ⁻¹ .d ⁻¹)	0.07	0.14	0.18	0.19	0.35	0.33	0.47	0.46	0.32	0.24

(1) The cycle length time (t_c) was 8 h (3 cycle/d) for all experimental conditions.

(2) Feed strategy was in batch (B, feeding time of 10 min, t_F/t_c=2%) or fed-batch (FB, 240 min, t_F/t_c=50%).

(3) The average percentage experimental error and average number of samples were, respectively, C_{ST} 14.3% (8 samples); C_{SF} 16.6% (8 samples); ε_{ST} 2.1% (8 samples); ε_{SF} 2.1% (8 samples); pH 2.5% (9 samples); TVA 22.8% (8 samples); BA 9.0% (8 samples); TS 8.7% (4 samples).

There are two factors that can cause the increase in productivity with the increase of organic load (or increase in concentration) under favourable environmental conditions: (i) the fact that the increase in organic load corresponds to the increase in the number of molecules that can be converted to methane; and (ii) an improvement in the reaction rate of substrate consumption by microorganisms. For organic loads higher than that experienced, if the yield maintains its decreasing pattern, then the methane production must decrease as well because the increase in substrate concentration is insufficient to maintain the rise in productivity.

With regard to the indicator Y_{M-CH₄/COD}, in Condition C08 (batch at 30°C, AVOL of 10.0 gCOD.L⁻¹.d⁻¹), the yield was 12.6 mmolCH₄.gCOD⁻¹; in Condition C09 (fed-batch at 30°C, AVOL of 10.0 gCOD.L⁻¹.d⁻¹), it was 11.8 mmolCH₄.gCOD⁻¹; and in Condition C10 (batch at 45°C, AVOL of 10.0 gCOD.L⁻¹.d⁻¹), it was 7.1 mmolCH₄.gCOD⁻¹. For the best experimental condition (Condition C07 – batch at 30°C, AVOL of 8.0 gCOD.L⁻¹.d⁻¹), the yield was 13.5 mmolCH₄.gCOD⁻¹. The ratios between the experimental yields for Conditions C07, C08, C09 and C10 and the maximum theoretical yield were 86%, 80%, 76% and 46%, respectively. For methane productivity, with a change in feed strategy to fed-batch mode and the rise in temperature to 45°C, fewer molecules of methane were produced by the same volume of reacting medium.

The maximum molar productivity achieved was in batch operation (Table 3) compared with fed-batch operation, with 102.5 molCH₄.m⁻³.d⁻¹, which corresponded to a volume of 2297 mL-STP-CH₄.L⁻¹.d⁻¹ and, with 45°C with 35.0 molCH₄.m⁻³.d⁻¹, which corresponded to a volume of 785 mL-STP-CH₄.L⁻¹.d⁻¹. This effect was probably due to a high reaction rate in batch mode in comparison to the dilution which occurred in fed-batch mode.

From Figure 2, one can observe that in Condition C08 (AVOL of 10.0 gCOD.L⁻¹.d⁻¹, batch at 30°C), the process of degradation likely reached a maximum threshold of methane productivity, but was already under the maximum yield. The transition to fed-batch in Condition C09 (AVOL of 10.0 gCOD.L⁻¹.d⁻¹, fed-batch at 30°C) was not sufficient to improve the yield and production of methane because the environmental condition was possibly close to the maximum threshold.

In addition to the decrease in the volume of biogas when the feed strategy was changed to fed-batch mode (Condition C08 to C09), when the temperature increased from 30°C to 45°C (Condition C08 to C10 – AVOL of 10.0 gCOD.L⁻¹.d⁻¹ and batch mode), this volume reduced drastically. This decrease could be due to the biomass loss observed during Condition C10, as indicated by an increase in total volatile solids in the effluent during the experiment (2254 to 3596 mgTVS.L⁻¹). The biomass loss could also be perceived visually from the grey/black colour of the material retained by the filter during effluent filtering.

Table 4 present the peaks of concentration of the intermediate compounds produced in a cycle for each experimental condition. Acetone and methanol concentration were below the limit of detection of the chromatographic method employed. The table indicates that, for an increasing organic load, in general, the following acids were prevalent: acetic, propionic and butyric. The maximum concentration of acetic and propionic acids in a cycle increased with increasing organic load, while the maximum concentration of butyric acid rose until Condition C03 and then decreased in the

subsequent conditions. Ethanol concentration was always low or lower than the limit of detection of until Condition C04 and then increased in Condition 05 due to the use of vinasse that had been previously frozen, which prevented ethanol degradation.

The change in feed strategy from batch mode to fed-batch mode (Condition C08) caused a decrease in the peaks of concentration in all intermediate compounds. This result was due to the characteristics of the fed-batch feed strategy because the influent was dosed in low quantities, which led to a decrease in the intermediate concentration.

Table 3. Performance indicators for all experimental conditions.

Parameter		C01	C02	C03	C04	C05	C06	C07	C08	C09	C10
		Increase in Applied Volumetric Organic Load (AVOL)									Fed-Batch
AVOL	(gCOD.L ⁻¹ .d ⁻¹)	1.1	2.1	3.0	4.1	5.2	6.2	8.2	10.1	9.7	10.3
X _{CH4}	(%)	67	76	76	76	79	78	79	78	73	65
X _{CO2}	(%)	33	24	24	24	21	22	21	22	27	35
Y _{M-CH4/COD}	(mmolCH ₄ .gCOD ⁻¹)	4.9	8.2	10.3	10.9	12.6	13.4	13.5	12.6	11.8	7.1
Y _{V-CH4/COD}	(mL-STPCH ₄ .gCOD ⁻¹)	109	184	230	244	283	301	302	281	265	160
Y _E /Y _T	(%)	31.2	52.5	65.8	69.6	80.7	85.8	86.2	80.3	75.6	45.7
MPr	(molCH ₄ .m ⁻³ .d ⁻¹)	4.8	15.7	28.0	40.7	63.5	81.0	107.2	123.4	102.5	35.0
SMP _r	(molCH ₄ .kgTVS ⁻¹ .d ⁻¹)	0.3	1.1	1.9	2.1	4.5	4.4	6.4	5.7	3.7	1.7
VPr	(mL-STPCH ₄ .L ⁻¹ .d ⁻¹)	107	352	627	911	1424	1815	2403	2767	2297	785
SVPr	(mL-STPCH ₄ .gTVS ⁻¹ .d ⁻¹)	7.7	25.7	42.3	46.6	100.4	99.2	143.0	128.3	83.9	38.4

(1) Theoretical yield of methane from consumed COD: 15.6 mmolCH₄.gCOD⁻¹ (molar) or 350 mLCH₄-STP.gCOD⁻¹ (volumetric).

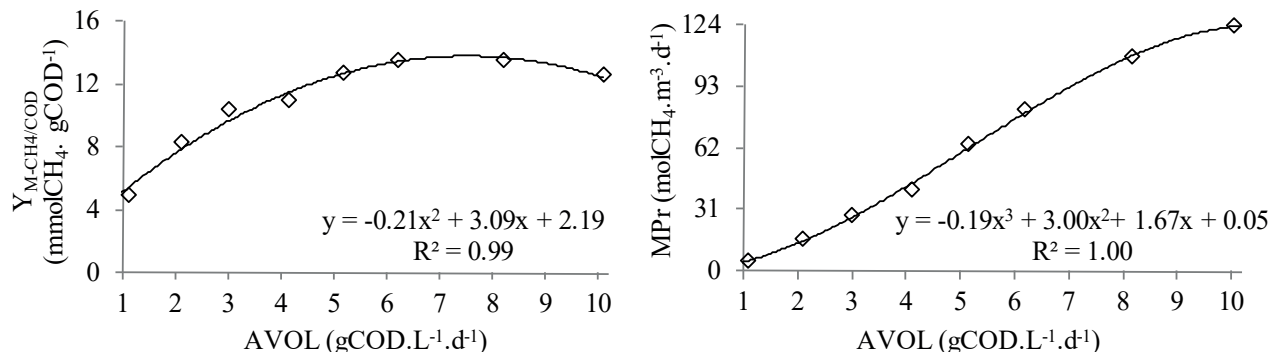


Figure 2. Evolution of the yield (Y_{M-CH4/COD}) and molar methane productivity indicators (MPr) with the applied volumetric organic load (AVOL).

Table 4. Maximum concentrations of the intermediate compounds (mg.L⁻¹) in a cycle for all experimental conditions *.

	Condition	EtOH	HAc	HPr	HBut	HVa	HCa
Increasing AVOL	C01	42	114	29	17	13	13
	C02	0	116	65	3	14	16
	C03	0	164	97	46	16	17
	C04	0	186	103	44	32	21
	C05	340	477	102	12	23	27
	C06	466	711	123	15	19	16
	C07	529	865	168	19	20	17
	C08	491	1207	176	9	13	16
Fed-Batch	C09	89	367	80	10	19	0
45°C	C10	50	5189	1351	14	15	15

* < Limit of detection estimated as 3 mg.L⁻¹.

In Condition C10, at 45°C, propionic acid predominated in relation to acetic acid. Thus, it is possible that acetogenic microorganisms were inhibited by temperature, thereby causing a conversion of propionic to acetic acid, which indicates a deviation in the main acetic metabolic pathway.

Analysis of the profiles along a cycle in batch mode (30°C)

Figures 3 and 4 show the profiles of organic matter concentration, TVA and methane for C01 to C08 (increasing in organic load) and C09 and C10 (change in feed strategy and temperature), as well as the model fitted to the experimental data. The visual analysis leads to the conclusion that the model could represent the tendencies of production and consumption of the presented compounds.

In Table 5, the first-order kinetic parameters for each experimental condition are presented, and in Figure 5, these parameters are presented for Conditions C01 to C08 as a function of organic load. In general, a similar tendency is found for both specific and non-specific kinetic parameters, which indicates that the changes in the environmental conditions are influenced in a similar manner by the individuals and the microbial population.

By analysing k_{IS} and k'_{IS} (Figure 5a), note that an increase in organic load led to a decrease in the organic matter consumption reaction rate in the transition of Condition C01 to C02. Next, considering k_{IS} , a tendency to increase with the increase in organic load (Conditions C02 to C08) is observed, whereas for k'_{IS} , a tendency for the reaction rate to decrease is observed. The k_{2M} and k'_{2M} generally presented a tendency to increase with load (Figure 5b). The same tendency can be observed for the molar productivity of methane (MP_r) (Table 3), i.e., the increase in the number of molecules produced in relation to the liquid medium is a result of the increase of the average reaction rate of methane production (C_{Mave}) observed in Conditions C01 to C08.

Considering the kinetic equation of the methane production rate, $r_M = k_{2M}(C_{TVA} - C_{TVAR})$, the increase in this reaction rate between the experimental conditions was due to both the increase of the average concentration of TVA, or the increase in substrate concentration, and the improvement in the production of molecules of methane by microorganisms, i.e., by the increase in the parameter k_{2m} (or k'_{2m}).

The parameters k_{1TVA} (or k'_{1TVA}) and k_{2TVA} (or k'_{2TVA}) define, respectively, the rate of production and consumption of TVA at each point of the profiles of TVA and of C_S . Based on the kinetic equation of rate of production or consumption of TVA [$r_{TVA} = k_{1TVA}(C_S - C_{SR}) - k_{2TVA}(C_{TVA} - C_{ATVA})$], the production term is due to the constant k_{1TVA} and concentration C_S , whereas the consumption term is due to the constant k_{2TVA} and the concentration C_{TVA} .

Figure 5e shows that the parameter k'_{2TVA} was higher

than k'_{1TVA} , thus revealing that consumption of acids was always higher than their production. The same can be said with regard to the non-specific parameters. Further, the increase in organic load led to a decrease in k'_{2TVA} relative to k'_{1TVA} so that the parameter values became similar in Condition 08 ($k'_{1TVA} = 0.0433 \text{ mg}^{-1} \cdot \text{L} \cdot \text{h}^{-1}$ and $k'_{2TVA} = 0.0585 \text{ mg}^{-1} \cdot \text{L} \cdot \text{h}^{-1}$). Thus, if this tendency is maintained in organic loads greater than that experienced when varying the influent concentration, the consumption term can eventually be overcome by the production term, ultimately leading the process to collapse due to the accumulation of acids.

These results are coherent with the indicators of productivity and yield of methane presented in Table 3. Further, they confirm that the process of degradation improved with the increase in organic load (only by increasing concentration) and validate the hypothesis that the process achieved a maximum level of degradation.

Analysis of the profiles along a cycle in fed-batch mode (45°C)

For Conditions C08, C09 and C10, based on an analysis of k_{IS} and k'_{IS} , Table 4 indicates that the reaction rate of substrate consumption decreased in fed-batch mode and when the temperature rose (transition between Conditions C08 and C09 and between C08 and C10). Regarding the parameters k_{2M} and k'_{2M} , the change in feed strategy led to an increase in methane production rate, with the kinetic parameters for Condition C09 being similar to those of Condition C08 in batch mode. However, the change in temperature led to a decrease in the rate of methane production.

Using the kinetic equation to determine the variation in TVA for fed-batch mode, [$C_{TVA} = (F/V)(C_{TVAf} - C_{TVA}) + k_{1TVA}(C_S - C_{SR}) - k_{2TVA}(C_{TVA} - C_{TVAR})$], it can be observed that the accumulation term is due to the constant k_{1TVA} , the concentration C_S and the TVA that are in the feed, whereas the consumption term is due to the constant k_{2TVA} and the concentration C_{TVA} . For fed-batch mode, because k_{2TVA} (or k'_{2TVA}) was higher than k_{1TVA} (or k'_{1TVA}), the microorganisms consumed more TVA than was produced. Thus, the accumulation of TVA observed in Figure 4 indicates that microorganisms were not able to consume TVA at a rate higher/equal to the rate at which TVA entered the reactor. The main TFA that enters the reactor is acetate, but Table 4 shows that propionate was the main intermediate that accumulated (~1350 mg/L) at 45°C; therefore, in this condition TVA were consumed at rates slower than they were produced.

In general, kinetic parameters for increasing temperatures indicate that the process as a whole was slower than observed at 30°C. Due to this increase, environmental conditions became unfavourable for some species of microorganisms, which gave privilege to species

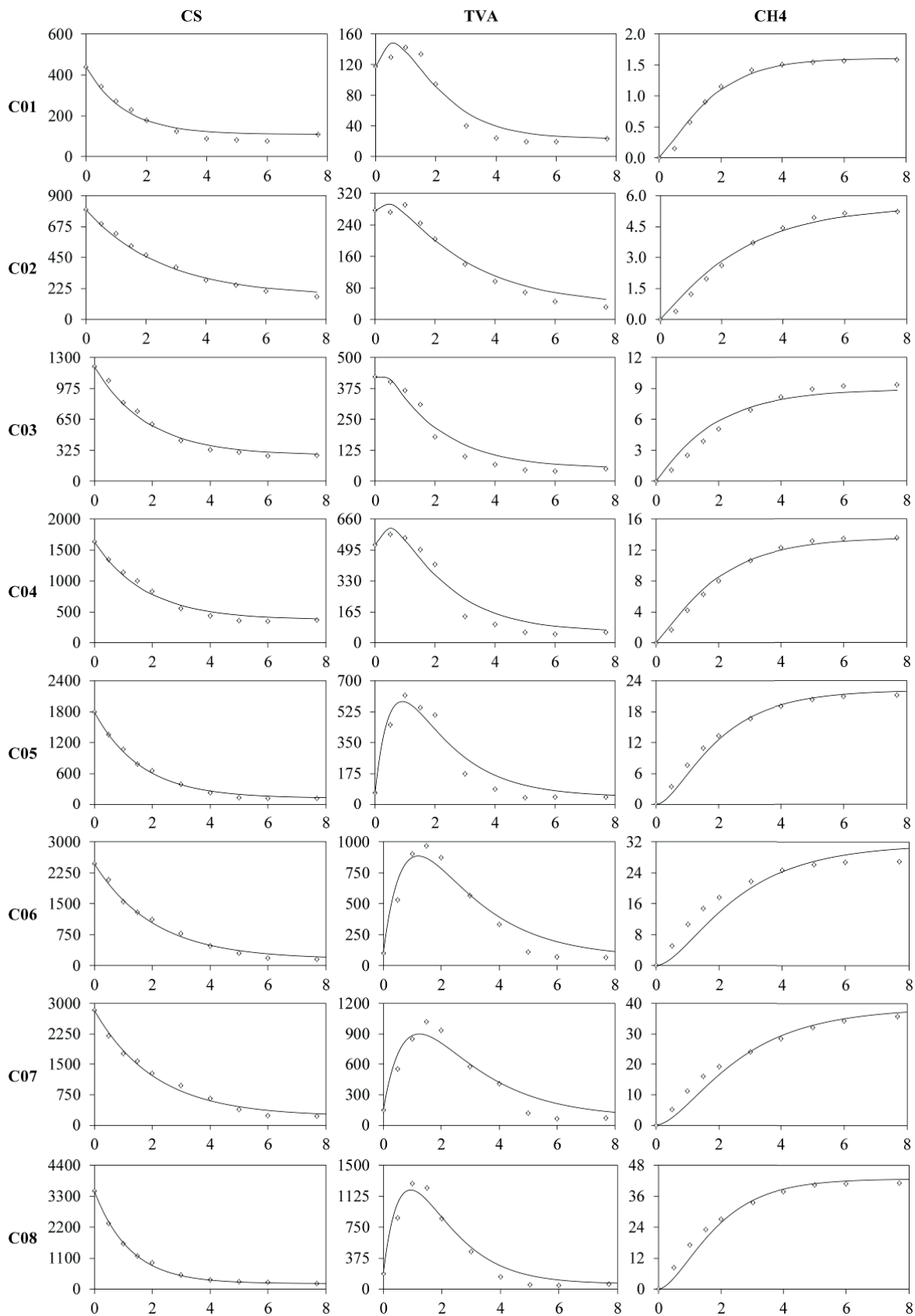


Figure 3. Model fitting (line) to experimental data (dots) for the profiles (8 h cycle time) of organic matter (CS), TVA and CH₄ for Conditions C01 to C08

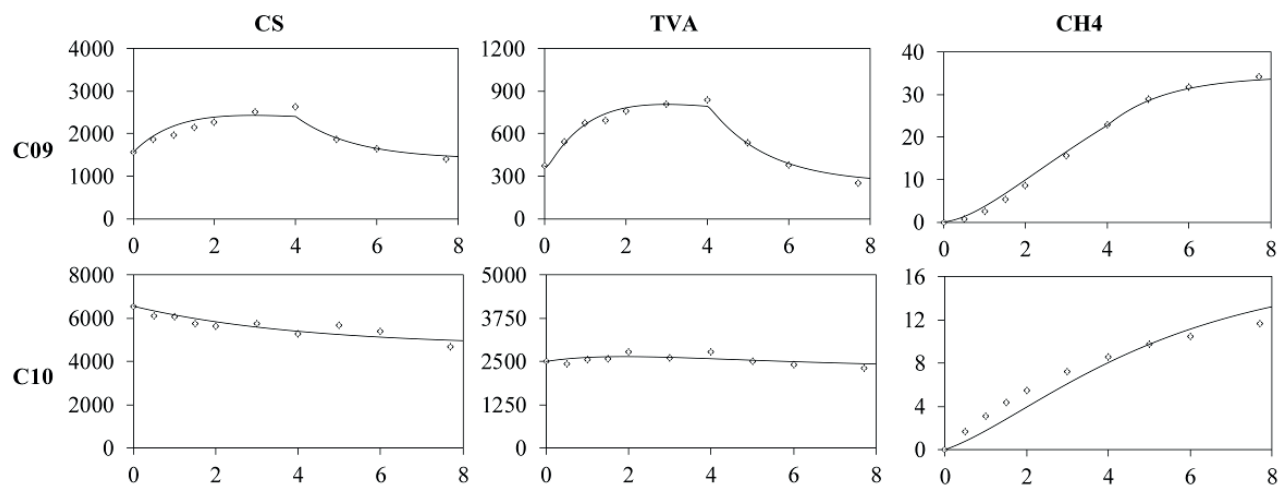


Figure 4. Model fitting (line) to experimental data (dots) for the profiles (cycle time of 8 h) of organic matter (CS), TVA and CH₄ for Conditions C09 and C10

Table 5. Kinetic parameters for all experimental conditions.

	C01	C02	C03	C04	C05	C06	C07	C08	C09 <i>Fed-Batch</i>	C10 <i>45°C</i>
	<i>Increase in Applied Volumetric Organic Load (AVOL)</i>									
C_{Sf}	-	-	-	-	-	-	-	-	10251	-
C_{S0}	438	797	1205	1633	1800	2473	2839	3496	1559	6559
C_{TVAf}	-	-	-	-	-	-	-	-	2026	-
C_{TVA0}	118	276	422	525	65	99	147	188	375	2500
C_{SR}	109	167	270	370	121	155	216	197	1400	4700
C_{TVAR}	23.0	32.0	50.0	55.0	42.0	63.0	72.0	63.0	250.0	2300.0
k_{IS}	0.784	0.387	0.548	0.560	0.617	0.482	0.477	0.816	0.693	0.245
k_{ITVA}	0.831	1.007	1.852	1.177	0.977	0.808	0.675	0.934	8.803	0.162
k_{2TVA}	1.450	2.108	4.028	2.102	1.728	1.273	1.182	1.261	16.50	0.556
k_{2M}	0.316	0.374	0.612	0.551	0.828	0.614	0.725	0.830	0.860	0.391
k'_{IS}	0.057	0.028	0.037	0.029	0.044	0.026	0.028	0.038	0.025	0.012
k'_{ITVA}	0.060	0.073	0.125	0.060	0.069	0.044	0.040	0.043	0.322	0.008
k'_{2TVA}	0.105	0.154	0.272	0.108	0.122	0.070	0.070	0.058	0.603	0.027
k'_{2M}	0.023	0.027	0.041	0.028	0.060	0.034	0.043	0.038	0.031	0.019

(1) Organic matter concentrations, biomass and solids in mg.L⁻¹;

(2) Kinetic parameters in h⁻¹ (non-specific) and mg⁻¹.L.h⁻¹ (specific).

that were better adapted. The temperature change to 45°C can be considered abrupt because there was insufficient time for biomass adaptation. Environmental conditions became severe because the reactor was subjected to a change in temperature at a high applied organic load.

At the end of Condition C09, microbial analysis was performed. The predominance of methanogenic bacilli can be observed, and their presence demonstrates that environmental conditions favourable to methanogens were achieved. *Methanosaeta* was also detected, which

is responsible for the assimilation of acetate (Garcia et al., 2000). The adherence of this microorganism could be favoured by the greater surface area created by the higher sponge porosity.

Comparison to other studies

The studies of Mockaitis et al. (2006), Selma et al. (2010), Lovato et al. (2012) and Silva et al. (2013) refer to the production of methane in *Anaerobic Sequencing Batch*

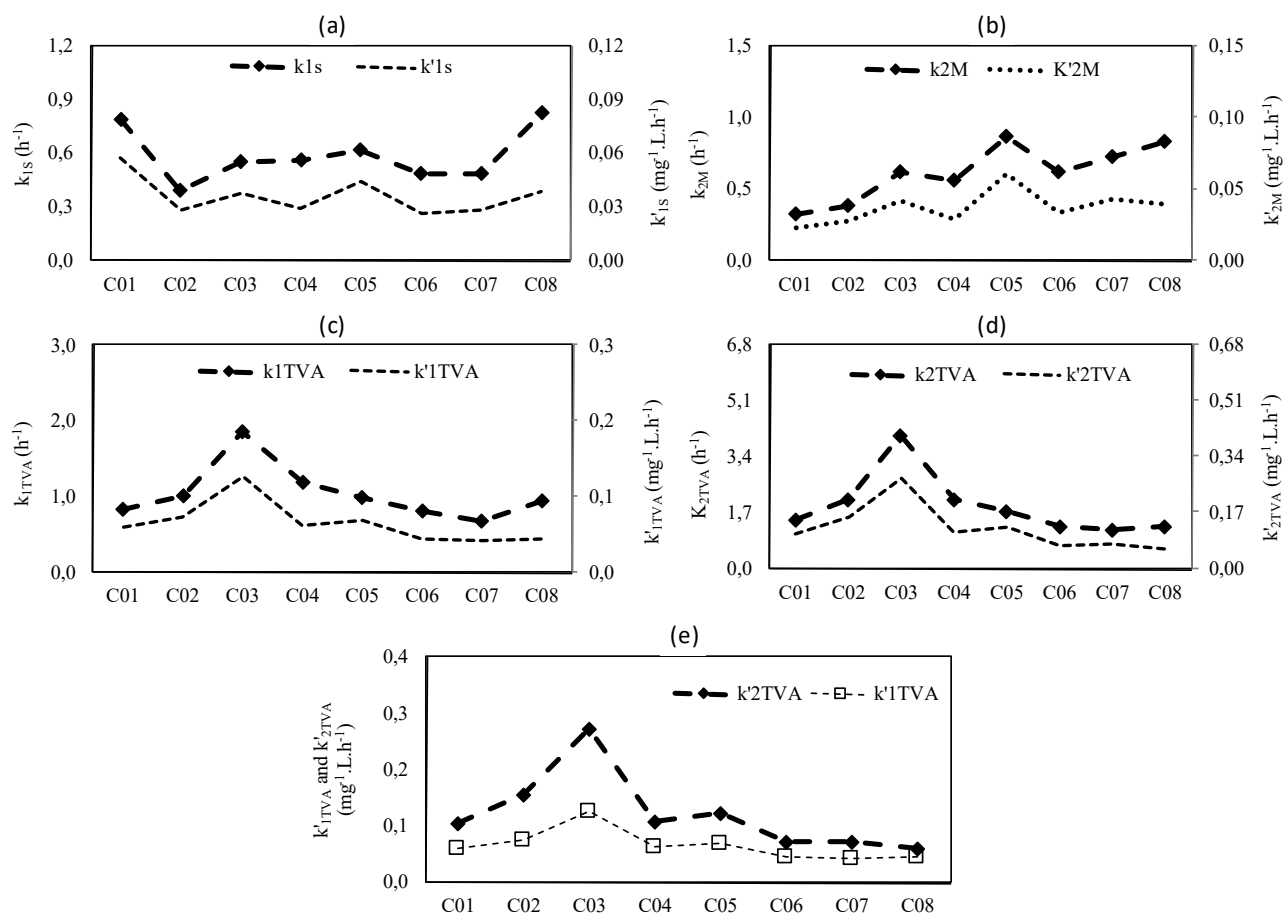


Figure 5. First-order apparent kinetic parameters for the organic matter consumption (k_{1s}), total volatile acids production (k_{1TVA}) and consumption (k_{2TVA}), and methane production (k_{2M}) and the relation between the specific kinetic parameters for production and consumption of TVA (k'_{1TVA} and k'_{2TVA}).

Biofilm Reactors (ASBR and AnSBBR), with either liquid phase recirculation (LPR) or mechanical stirring (MS), applied to the treatment of other wastewaters (whey and glycerine). These studies and the present paper assessed experimental conditions with cycle times of 4h and 8h, volume of reactor of 3,0 L or 5,0 L and feed strategy of batch and fed-batch. In the present study the applied volumetric organic load covered a wide range of values, and this made possible the comparison with the other papers.

Mockaitis et al. (2006) treated 2 L of whey per cycle with an ASBR (MS). The AVOL was in the range of 1.15 gCOD.L⁻¹.d⁻¹ to 4.79 gCOD.L⁻¹.d⁻¹. The maximum values of yield, volume and percentage of methane in biogas were, respectively, 5.3 mmolCH₄.gCOD⁻¹, 908 NmLCH₄ and 61%. Selma et al. (2010) treated 2 L per cycle of an effluent of biodiesel (glycerine) produced in laboratory with an ASBR (MS). The AVOL was in the range of 0.60 gCOD.L⁻¹.d⁻¹ to 3.82 gCOD.L⁻¹.d⁻¹. The maximum values of yield, concentration, volume and percentage of methane in biogas were, respectively, 11.8 mmolCH₄.gCOD⁻¹, 11.8 mmol.L⁻¹ biogas, 232 NmLCH₄ and 74%. Lovato et al. (2012) treated 1.5 L per cycle of an effluent

of biodiesel (glycerine) produced in laboratory with an AnSBR (LPR). The AVOL was in the range of 4.5 gCOD.L⁻¹.d⁻¹ to 7.5 gCOD.L⁻¹.d⁻¹. The maximum values of yield, concentration, and percentage of methane in biogas were, respectively, 5.3 mmolCH₄.gCOD⁻¹, 13.5 mmol.L⁻¹ biogas and 75%. Finally, Silva et al. (2013) treated 1 L and 2 L per cycle of an effluent of biodiesel (glycerine) produced in industry with an ASBR (MS). The AVOL was in the range of 1.23 gCOD.L⁻¹.d⁻¹ to 3.89 gCOD.L⁻¹.d⁻¹. The maximum values of yield, concentration, volume and percentage of methane in biogas were, respectively, 11.9 mmolCH₄.gCOD⁻¹, 13.9 mmol.L⁻¹ biogas, 319 NmLCH₄ and 74%.

In general, it can be noted that the ASBR reactor with mechanical stirring treating vinasse presented a better performance than the other reactors treating effluent of biodiesel production (industrial and laboratorial) and whey. In the other studies the yield of methane reached approximately 12 mmolCH₄.gCOD⁻¹, but this value is related to a small AVOL. In the present study the maximum yield of methane (13.5 mmolCH₄.gCOD⁻¹) is related to an AVOL of 6 to 13 times greater. Besides, the increase in AVOL produced an increase in the yield of methane up

to the maximum, while in the other reactors a decrease is generally observed.

In addition to the comparison, it can be noted in the literature that there are some studies assessing vinasse treatment using other reactors, such as UASB and USBF (UASB + anaerobic filter). Molina et al. (2007) treated wine vinasse using a hybrid anaerobic reactor (USBF) on a pilot scale with AVOL of 12 gCOD.L⁻¹.d⁻¹ (in comparison to 10 gCOD.L⁻¹.d⁻¹ of the present study) and they obtained a COD removal of 96% and a percentage of methane in biogas of 74%, values that are smaller than that of the present study (98% e 78%, respectively). On the other hand, España-Gamboa et al. (2012) treated vinasse from hydrous ethanol using a modified UASB reactor with an AVOL of 17 gCOD.L⁻¹.d⁻¹ and obtained COD removal of 69%, a value that is smaller than that of the present study. These researchers, in turn, obtained a better percentage of methane in biogas, which was 84%.

CONCLUSIONS

With an increase in the applied volumetric organic load (AVOL) from 1.1 to 10.1 gCOD.L⁻¹.d⁻¹, the methane productivity increased up to 123.4 molCH₄.m⁻³.d⁻¹ to reach a maximum yield of 13.5 mmolCH₄.gDQO⁻¹ in 8.2 gCOD.L⁻¹.d⁻¹ before decreasing at an AVOL of 10.1 gCOD.L⁻¹.d⁻¹. In fed-batch operation, these parameters were smaller (max.: 102.5 molCH₄.m⁻³.d⁻¹ and 11.80 mmolCH₄.gDQO⁻¹), and at 45°C, all of the parameters were reduced (max.: 35.0 molCH₄.m⁻³.d⁻¹ and 7.10 mmolCH₄.gDQO⁻¹). In general, the methane yield and productivity, the kinetic parameters and the overall factors of the degradation process presented here lead to the conclusion that AnSBBR

performed well in treating vinasse for applied organic loads up to 10.0 gCOD.L⁻¹.d⁻¹ and for concentration up to 10.0 gCOD.L⁻¹.

Thus, it can be noted that the AnSBBR applied to the treatment of vinasse performed better than other batch reactors applied to the treatment of other wastewaters (whey and glycerine), such as ASBR and AnSBBR that employed liquid phase recirculation. On the other hand, it presented similar performance when compared to continuous reactors, such as UASB, achieving better values for some performance parameters.

The fitting of the proposed model to the data and accumulation of intermediate compounds indicate how the process was dependent of the environmental conditions (AVOL), that could become favourable for some microorganisms and unfavourable for others less adapted. Furthermore, a temperature change should be considered only after sufficient time for biomass adaptation.

Further research in the mesophilic range may focus on the assessment of the effects of changing in the organic load on the process by the variation of the ratio between the feed volume and the reactor volume (V_F/V_R) and by the number of daily cycles (N). Also, it can be suggested that, in the thermophilic range, the effects of changing the organic load on the process be studied by the variation of the influent concentration (C_{SINF}), the ratio V_F/V_R and the number of daily cycles (N).

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NOMENCLATURE

Abbreviation/ symbol	Denomination	Unit
AnSBBR	Anaerobic Sequencing Batch Biofilm Reactor	-
ASBR	Anaerobic Sequencing Batch Reactor	-
COD	Chemical oxygen demand	(mgCOD.L ⁻¹)
V_R	Volume of liquid into the reactor	(L)
V_F	Volume of wastewater fed per cycle	(L.cycle ⁻¹)
N	Number of cycles per day	(cycle.d ⁻¹)
t_C	Time cycle	(h)
t_F	Feeding time	(min)
C_{SINF}	Organic matter concentration in influent	(mgCOD.L ⁻¹)
C_{SF}	Organic matter concentration in feed	(mgCOD.L ⁻¹)
C_{S0}	Filtered organic matter concentration at initial time of the cycle	(mgCOD.L ⁻¹)
C_{ST}	Non-filtered organic matter concentration	(mgCOD.L ⁻¹)
C_{SF}	Filtered organic matter concentration	(mgCOD.L ⁻¹)
ϵ_{ST}	Efficiency of removal of non-filtered organic matter	(%)
ϵ_{SF}	Efficiency of removal of filtered organic matter	(%)
AVOL	Applied volumetric organic load, expressed by COD	(gCOD.L ⁻¹ .d ⁻¹)
ASOL	Applied specific organic load, expressed by COD	(gCOD.gTVS ⁻¹ .d ⁻¹)

RVOL	Removed volumetric organic load, expressed by COD	(gCOD.L ⁻¹ .d ⁻¹)
RSOL	Removed specific organic load, expressed by COD	(gCOD.gTVS ⁻¹ .d ⁻¹)
BA	Bicarbonate alkalinity	(mgCaCO ₃ .L ⁻¹)
TVA	Total volatile acids	(mgHAc.L ⁻¹)
Acetone	Acetone concentration	(mg.L ⁻¹)
MetOH	Methanol concentration	(mg.L ⁻¹)
EtOH	Ethanol concentration	(mg.L ⁻¹)
ButOH	N-butanol concentration	(mg.L ⁻¹)
HAc	Acetic acid concentration	(mg.L ⁻¹)
HPr	Propionic acid concentration	(mg.L ⁻¹)
HIsoBut	Isobutyric acid concentration	(mg.L ⁻¹)
HBut	Butyric acid concentration	(mg.L ⁻¹)
HIsoVal	Isovaleric acid concentration	(mg.L ⁻¹)
HVal	Valeric acid concentration	(mg.L ⁻¹)
HCa	Caproic acid concentration	(mg.L ⁻¹)
TS	Total solids	(mgTS.L ⁻¹)
TVS	Total volatile solids	(mgTVS.L ⁻¹)
TSS	Total suspended solids	(mgTSS.L ⁻¹)
VSS	Volatile suspended solids	(mgVSS.L ⁻¹)
FSS	Fixed suspended solids	(mgFSS.L ⁻¹)
TDS	Total dissolved solids	(mgTDS.L ⁻¹)
VDS	Volatile dissolved solids	(mgVDS.L ⁻¹)
M _{TVS}	Mass of total volatile solids in the reactor	(g)
C _X	Mass of total volatile solids per volume of liquid medium	(g.L ⁻¹)
C _{X'}	Mass of total volatile solids per mass of inert support	(g.g ⁻¹)
V _G	Volume of biogas at STP	(mL-STP.cycle ⁻¹)
V _{CH4}	Volume of methane at STP	(mL-STP-CH ₄ .cycle ⁻¹)
N _{CH4}	Moles of methane produced	(molCH ₄ .cycle ⁻¹)
X _{CH4}	Percentage of methane in biogas	(%)
X _{CO2}	Percentage of carbonic gas in biogas	(%)
Y _{M-CH4/DQO}	Molar yield of methane from consumed organic matter	(mmolCH ₄ .gCOD ⁻¹)
Y _{V-CH4/COD}	Volumetric yield of methane from consumed organic matter	(mL-STP-CH ₄ .gCOD ⁻¹)
MPr	Mol of methane per day and per reactor volume	(molCH ₄ .m ⁻³ .d ⁻¹)
SMPr	Mol of methane per day and per reactor TVS	(molCH ₄ .kgTVS ⁻¹ .d ⁻¹)
VPr	Volume of methane per day and per reactor volume	(mL-STP-CH ₄ .L ⁻¹ .d ⁻¹)
SVPPr	Volume of methane per day and per reactor TVS	(mL-STP-CH ₄ .gTVS ⁻¹ .d ⁻¹)

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