

THERMOPHILIC BIOMETHANE PRODUCTION BY VINASSE IN AN AnSBBR: START-UP STRATEGY AND PERFORMANCE OPTIMIZATION

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Abstract - Thermophilic anaerobic digestion treatment of industrial wastewater generated at high temperatures can be used as an alternative for environmental pollution control and bioenergy production. Therefore, this study investigated thermophilic anaerobic reactors containing immobilized biomass (AnSBBR) to treat vinasse for methane production, in batch and fed-batch mode, in a three-step approach. In *Step I* (batch), the biomass was properly adapted to the thermophilic condition (55 °C) with a feed containing vinasse plus molasses as a co-substrate. In *Step II* (batch), the applied volumetric organic load (AVOL) was increased and resulted in a methane molar productivity of 304 molCH₄.m⁻³.d⁻¹ at an AVOL of 25.9 gCOD.L⁻¹.d⁻¹ and a yield of methane per removed organic matter near 331 NmL-CH₄.gCOD⁻¹. In *Step III*, a fed-batch strategy was employed at an AVOL of 25.1 gCOD.L⁻¹.d⁻¹ and achieved an optimum methane productivity of 352 molCH₄.m⁻³.d⁻¹ with COD and carbohydrate removal efficiencies of approximately 80 % and 90%, respectively. A kinetic model fitted to the experimental data allowed better understanding of the anaerobic metabolic reactions. Finally, the results obtained demonstrated that a thermophilic AnSBBR is an efficient technological alternative for methane production through vinasse digestion. **Keywords:** Methane; Feed time; Influent concentration; Anaerobic reactor.

INTRODUCTION

Environmental problems inherent in the exploration and use of fossil fuels have stimulated the development of biofuels, such as ethanol, which can be obtained from different sources (Wilkie et al, 2000; Gamboa et al., 2011). In Brazil ethanol, of which the production exceeded 30 billion liters in 2016 (CONAB, 2017), is obtained mainly from sugarcane, as climate and territorial extension facilitate cultivation of this crop. An inherent problem faced by ethanol and sugar mills is the destination of the by-product vinasse, which is generated at a ratio of 12 - 15 liters per liter of produced ethanol. The alternative most adopted for

vinasse destination is soil fertigation, i.e., vinasse is incorporated into the water used for irrigation, which in the long run may cause changes in the physical properties of the soil.

This problem, allied to the growing volume of generated vinasse, has stimulated the search for technologies that reduce the polluting potential of vinasse without losing its nutritive properties. One of these technologies is the anaerobic reactor operated in batch and fed-batch mode (AnSBBR) which has presented promising results regarding environmental compliance and energy recovery from wastewaters. These reactors are indicated for wastewaters with high organic load and nutritive characteristics, such

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as vinasse, because nutrient properties are conserved even after treatment, which enables further use as fertilizer (Vlissidis and Zouboulis, 1993; Barros et al., 2016).

In the literature, few studies have focused on the thermophilic treatment of vinasse in anaerobic reactors and the settings that offer the best performance and operational stability have not been well defined yet. Ribas et al. (2009) reported 70 % COD removal and a 70 % methane fraction in biogas by a mesophilic AnSBBR reactor for sugar cane-vinasse treatment. Almeida et al. (2017) studied the same reactor configuration and reported an increase in COD removal efficiency (97 %) and methane productivity ($123 \text{ molCH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$) with increasing applied volumetric organic load (AVOL) ($1.1 - 10.1 \text{ gCOD} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$). It should be mentioned that a major challenge in the vinasse anaerobic treatment is temperature. During the distillation process the liquid achieves temperatures near 90°C , which reduces to 60°C on the way to the treatment system due to heat losses. To render thermophilic treatment practicable for this wastewater, cooling should be used to apply biologic treatment under mesophilic conditions (Ribas et al., 2009).

Within this context, this investigation aimed to study the biotechnological viability of an anaerobic

reactor operating in batch and fed-batch mode with mechanical stirring and immobilized biomass (AnSBBR) under thermophilic conditions treating vinasse for methane production. To this end, an assessment was made of the effect of the thermophilic biomass adaptation strategy on vinasse, as well as of the effect of increasing organic load in batch and fed-batch mode on performance indicators related to operational stability, organic matter removal, yield (methane generated per substrate consumed) and biogas productivity/composition.

MATERIALS AND METHODS

AnSBBR

The anaerobic sequencing batch biofilm reactor containing immobilized biomass used in this work was the same as that of Albanez et al. (2016). The AnSBBR (Figure 1) was operated in batch and fed-batch mode, at 55°C , controlled by a water jacket, of which the temperature was regulated by an ultrathermostatic bath. Stirring was set at 100 rpm and the cycle length was 8 h.

Inoculum, inert support and feed composition

The inoculum, taken from a thermophilic UASB reactor treating vinasse from an ethanol plant, contained

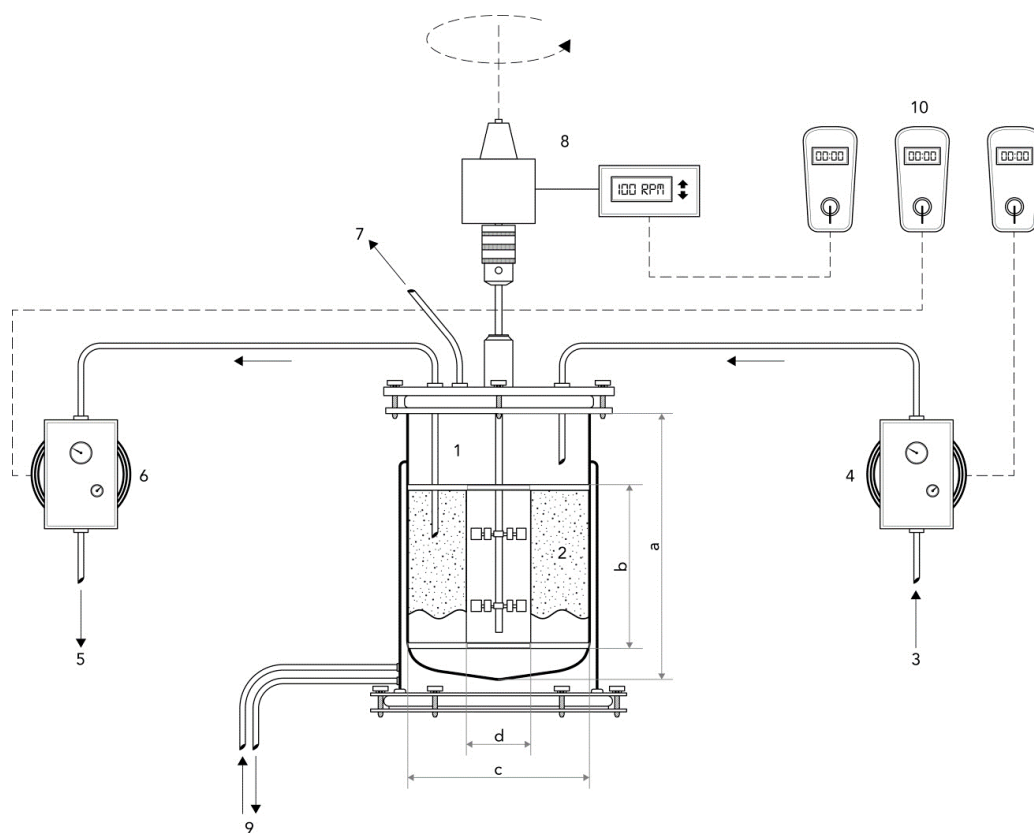


Figure 1. Scheme of the reactor used in this study. [(a) Reactor BIOFLO III (New Brunswick Scientific Co.) with 6 L capacity; 2 – basket containing support material with immobilized biomass ($c = 18.0 \text{ cm}$; $d = 7.0 \text{ cm}$); 3 – Influent; 4 – Feed pump; 5 – Effluent; 6 – Discharge pump; 7 – Biogas outlet; 8 – Mechanical agitation; 9 – Temperature control system (ultrathermostatic bath)]. Adapted from Albanez et al. (2016)

17 g.L⁻¹ of total volatile solids. The inert support used to immobilize the biomass consisted of polyurethane foam cubes (1 cm edges). The immobilization process was performed according to the method proposed by Zaiat et al. (1994).

The molasses and vinasse used to prepare the wastewater were obtained from a bioethanol plant and they were stored at -4 °C. The vinasse presented 27.6 ± 4.6 gCOD.L⁻¹ (6.2 ± 1.8 gCarbohydrate.L⁻¹) and pH 4.5, whereas the molasses presented an average of 1.2 gCOD.L⁻¹.

According to each experimental stage, the wastewater was diluted with water from the public supply system and supplemented with urea (5.8 mgCH₄N₂O.L⁻¹ per 1000 mgCOD.L⁻¹), as a nitrogen source, and sodium bicarbonate (200 - 100 mg NaHCO₃.L⁻¹ per 1000 mgCOD.L⁻¹) as a buffering agent. These values were proportionally modified according to the effluent concentration.

Physical-chemical analyses

Reactor monitoring was carried out for influent and effluent samples at least 4 times a week, except for the solids analyses which were carried out twice. The reactor performance was monitored as Chemical Oxygen Demand (COD - C_{MO}) and Carbohydrate (C_C) in filtered samples (C_{MOF} and C_{CF} - filtered on a membrane of micro glass fiber with nominal pore diameter of 0.45 µm). The system stability was monitored by measuring pH, bicarbonate alkalinity (BA), total volatile acids (TVA), total solids (TS), total volatile solids (TVS), total suspended solids (TSS) and volatile suspended solids (VSS). The analyses were performed according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995), Ripley et al. (1986) for alkalinity determination, and Dubois et al. (1956) for carbohydrate determination.

The intermediate compounds analyses of the anaerobic metabolism (ethanol and organic acids: acetic, propionic, butyric/isobutyric, valeric/isovaleric and caproic; limit of detection of 0.5 mmol.L⁻¹) was performed via gas chromatography (head-space) with external standard (iso-butanol and crotonic acid) using an Agilent ® 7890 chromatograph equipped with a flame ionization detector and an HPInnowax column. The biogas composition (carbon dioxide - CO₂ and methane - CH₄; limit of detection of 0.1 mmol.L⁻¹) was also performed by gas chromatography using the same chromatograph but equipped with a thermal conductivity detector and GS-Carbonplot column. These methods are described in Almeida et al. (2017).

Stability and performance indicators

The stability and performance indicators used are described in Almeida et al. (2017): removal efficiency of total organic matter for soluble samples (ε_p), applied

volumetric organic load (AVOL), removed volumetric organic load (RVOL), molar (MPr) and volumetric (VPr) productivity, specific molar (SMPPr) and specific volumetric (SVPr) productivity and methane yield per consumed load (Y_{MO}).

The biogas volume produced during a cycle (V_G) was measured using a Ritter® MilligasCounter. The accumulated volumes of biogas at each point were calculated by Equation (1) for the fed-batch mode condition (V_{Gi-BA}), in which the quantification was corrected because the biogas production occurred concomitant with the effluent feeding to the reactor and by Equation (2) for the batch mode condition (V_{Gi-B}), using the volume recorded by the biogas meter (V_{M-i}), the biogas profile point number (N_i), the total number of profile points during the fed batch (N_{t-BA}) and effluent volume fed during the cycle (V_A).

$$V_{Gi-BA} = V_{M-i} - \frac{N_i}{N_{t-BA}} V_A \quad (1)$$

$$V_{Gi-B} = V_{M-i} - V_A \quad (2)$$

The conversion of the biogas volume at standard temperature and pressure conditions was calculated according to the general gas law by Equation (3) and the number of moles of generated methane (n_{CH₄} in mmol) was calculated by Equation (4), using volume (V_N) at STP, the biogas volume to be converted (V_i - V_{Gi-BA} or V_{Gi-B}), air pressure at the measurement point (P_A), partial pressure of water vapor (P_v), pressure of the liquid column above the measuring chamber (P_L = 2 mbar), normal pressure (P_N = 1013.25 mbar), normal temperature (T_N = 273.15 K), temperature inside the reactor (T_a = 55 °C), pressure (P = 1 atm) and Clapeyron constant (R = 0.082 atm.L.K⁻¹.mol⁻¹).

$$V_N = V_i \frac{(P_A - P_v + P_L)}{P_N} \frac{T_N}{T_a} \quad (3)$$

$$n_{CH_4} = \frac{P \cdot V_N}{R \cdot T_N} \quad (4)$$

At the end of each experimental condition, the reactor was discharged and the residual volume (V_R) of liquid medium in the reactor was measured. The inert support holding immobilized biomass was weighed and a sample of this material (foam and biomass) was collected and quantified. It should be mentioned that there is no guarantee that TVS consists only of biomass, due to the complex composition of the medium and the measurements are hence only an estimate. Next, the foam was washed with distilled water, which separated the solid (inert support) and the liquid (immobilized biomass) phases. TS was measured in the solid phase,

and TS (M_{A-TS}) and TVS (M_{A-TVS}) were measured in the liquid phase. The total amount of biomass in the reactor (M_{TVS}) was calculated by Equation (5), where (M_{T-SI+B}) is total biomass and foam inside the reactor, (M_{A-TVS}) is the mass of total volatile solids in the sample and (M_{A-SI+B}) is the sample collected from the inert material containing immobilized biomass.

The relation between the amount of biomass and liquid medium in the reactor (C_X) was calculated by Equation (6), where V_R is the liquid volume in the reactor, and the relation between the amount of biomass and inert support in the reactor ($C_{X'}$) was calculated by Equation (7), where M_{A-SI} is the amount of inert support available for immobilization.

$$M_{TVS} = \frac{M_{A-TVS} \cdot M_{T-SI+B}}{M_{A-SI+B}} \quad (5)$$

$$C_X = \frac{M_{TVS}}{V_R} \quad (6)$$

$$C_{X'} = \frac{M_{A-TVS}}{M_{A-SI}} \quad (7)$$

Experimental Procedure

The operating procedure of the reactor was as follows: a) feeding - 1.0 L in 10 min in batch mode and 240 min in fed-batch mode; b) agitation and reaction (for fed-batch mode the feed time was included); c) discharge - 1.0 L in 10 min, guaranteeing a residual volume (V_R) of 1.3 L of the total liquid volume inside the reactor (2.3 L). In all experiments the AnSBBR was operated at 55 °C, stirring was set at 100 rpm with a cycle length of 8 h. These values were adopted from previous investigations of methane production using the reactor (Almeida et al., 2017; Albanez et al., 2016; Silva et al., 2013; Lovato et al., 2012; Bezerra et al., 2011; Selma et al., 2010; Michelan et al., 2009).

The reactor operation was performed in three steps:

- **Step I** (duration of 40 days): The reactor was operated in batch mode and the assays were carried out in two phases to implement a reactor start-up strategy that allows acclimatization of the biomass. Phase 1: wastewater was molasses-based because of its high biodegradability. The influent concentration and temperature were increased from 1000 mgCOD.L⁻¹ to 5000 mgCOD.L⁻¹ (1.5 – 7.0 gCOD.L⁻¹.d⁻¹) and from 35 °C to 55 °C, respectively. They were modified as soon as the monitored parameters achieved stability. Phase 2: concentration (5000 mgCOD.L⁻¹) and temperature (55 °C) were kept constant and wastewater composition was altered by adding vinasse (from 100 % molasses to 100 % vinasse).

- **Step II** (duration of 110 days): The reactor was operated in batch mode, at 55 °C, with vinasse-

based wastewater, using different volumetric organic loads by varying the influent concentration from 5000 mgCOD.L⁻¹ to 20000 mgCOD.L⁻¹ (AVOL 6.5 to 25.9 gCOD.L⁻¹.d⁻¹);

- **Step III** (duration of 25 days): The reactor was operated at 55 °C, the wastewater was vinasse-based, influent concentration was 20000 mgCOD.L⁻¹ (25.1 gCOD.L⁻¹.d⁻¹), and the feeding strategy was modified to fed-batch mode with feeding time of 240 min.

The mean values of the monitored variables were calculated for each experimental condition as follows: (i) 14 samples for each of the operational variables: temperature, liquid volume (fed per cycle), substrate (COD and Carbohydrate), bicarbonate alkalinity, total volatile acids, biogas production and composition (CH₄/CO₂) resulting in a maximum standard deviation of 6%; (ii) 6 samples for each of the operational variables: influent/effluent solids (TS, TSS, VSS) resulting in a maximum standard deviation of 12%; (iii) 2 samples for each of the operational variables: (HAc, HPr, HBu, HVa) and biomass inside the reactor (C_X), resulting in a maximum standard deviation of 8%.

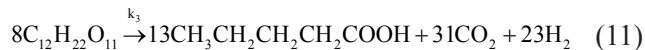
When reactor stability was achieved under the monitored experimental conditions, a time profile was taken along the operating cycle regarding soluble organic matter concentrations (in the form of COD and carbohydrate), bicarbonate alkalinity, pH, total volatile acids, intermediate metabolites and biogas (composition and production). Samples were taken every 30 to 60 min, in a way to not exceed 200 mL of total collected volume. In this way, it was possible to get a better understanding of the metabolic routes along the cycle. Thus, a new experimental condition was implemented by changing the influent concentration or the feeding strategy.

Kinetic metabolic model

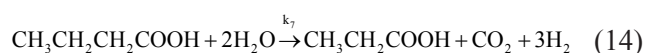
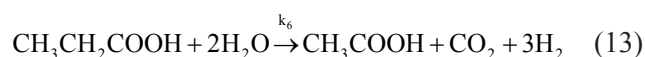
The kinetic model for the metabolic pathways proposed in this investigation was adapted from Bagley and Brodtkorb (1999), Rodrigues et al. (2004) and Lovato et al. (2016). The model admits a simplified metabolic pathway in eleven steps (Equations 8 to 18). In the first five parallel steps (*hydrolysis and acidogenesis*), the substrate (S), sucrose, was converted to acetic acid (HAc), propionic acid (HPr), butyric acid (HBu), valeric acid (HVa) and ethanol (EtOH). In the following four steps (*acetogenesis*), propionic acid, butyric acid, valeric acid and ethanol were consumed to acetic acid, shorter chain acids and hydrogen (H). In the next two independent steps (*methanogenesis*), methane (M) was produced by acetoclastic and hydrogenotrophic routes. At all stages, conversion reactions were considered to be first order.

Hydrolysis and acidogenesis

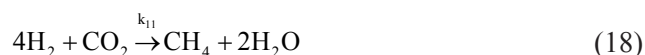




Acetogenesis



Methanogenesis



Equations (19) to (26) present the reaction rate equations for consumption of substrate (r_s), consumption and/or formation of acetic acid (r_{HAc}), propionic acid (r_{HPr}), butyric acid (r_{HBu}), valeric acid (r_{HVa}), ethanol (r_{EtOH}), hydrogen (r_H), and methane (r_M), respectively, containing apparent kinetic parameters associated with substrate consumption, volatile acids formation/consumption and methane formation. The kinetic parameter “k” refers to reaction rate constant, indicating a relation with the time required for the compound concentration (S, HAc, HPr, HBu, HVa, EtOH, H and M) to reach a residual value according to the kinetic model hypothesis. Indices “1 to 11” refer to the reactions and indices “S, HAc, HPr, HBu, HVa, EtOH, H and M” refer to the experimental values used to calculate these parameters.

$$r_s = -(k_{1S} + k_{2S} + k_{3S} + k_{4S} + k_{5S}) \cdot C_S = -k'_{1S} \cdot C_S \quad (19)$$

$$r_{HAc} = k_{1HAc} \cdot C_S + k_{6HAc} \cdot C_{HPr} + k_{9HAc} \cdot C_{EtOH} - k_{10HAc} \cdot C_{HAc} \quad (20)$$

$$r_{HPr} = k_{2HPr} \cdot C_S - k_{6HPr} \cdot C_{HPr} + k_{7HPr} \cdot C_{HBu} \quad (21)$$

$$r_{HBu} = k_{3HBu} \cdot C_S - k_{7HBu} \cdot C_{HBu} + k_{8HBu} \cdot C_{HVa} \quad (22)$$

$$r_{HVa} = k_{4HVa} \cdot C_S - k_{8HVa} \cdot C_{HVa} \quad (23)$$

$$r_{EtOH} = k_{5EtOH} \cdot C_S - k_{9HAc} \cdot C_{EtOH} \quad (24)$$

$$r_H = k_{1H} \cdot C_S - k_{2H} \cdot C_S + k_{3H} \cdot C_S + k_{4H} \cdot C_S + k_{6H} \cdot C_{HPr} + k_{7H} \cdot C_{HBu} + k_{8H} \cdot C_{HVa} + k_{9H} \cdot C_{EtOH} - k_{11H} \cdot C_H \quad (25)$$

$$r_M = k_{10M} \cdot C_{HAc} + k_{11M} \cdot C_H \quad (26)$$

Equations (27) to (35) present the mass balance of the reactor in batch mode ($F=0$) and fed-batch mode ($F \neq 0$) regarding the kinetic model (substrate, volatile acids and methane; subscripts “INF” are related to concentration of the compounds in the influent). These equations were used to determine the kinetic parameters of the model.

$$\frac{dV}{dt} = F \quad (27)$$

$$\frac{dC_S}{dt} = \frac{F}{V} \cdot (C_{S\ INF} - C_{S\ F}) + r_s \quad (28)$$

$$\frac{dC_{HAc}}{dt} = \frac{F}{V} \cdot (C_{HAc\ INF} - C_{HAc}) + r_{HAc} \quad (29)$$

$$\frac{dC_{HPr}}{dt} = \frac{F}{V} \cdot (C_{HPr\ INF} - C_{HPr}) + r_{HPr} \quad (30)$$

$$\frac{dC_{HBu}}{dt} = \frac{F}{V} \cdot (C_{HBu\ INF} - C_{HBu}) + r_{HBu} \quad (31)$$

$$\frac{dC_{HVa}}{dt} = \frac{F}{V} \cdot (C_{HVa\ INF} - C_{HVa}) + r_{HVa} \quad (32)$$

$$\frac{dC_{EtOH}}{dt} = \frac{F}{V} \cdot (C_{EtOH\ INF} - C_{EtOH}) + r_{EtOH} \quad (33)$$

$$\frac{dC_H}{dt} = \frac{F}{V} \cdot (C_{H\ INF} - C_H) + r_H \quad (34)$$

$$\frac{dC_M}{dt} = -\frac{F}{V} \cdot (C_M) + r_M \quad (35)$$

To deal with the differential equation, the Euler numerical integration method (Excel® software) was used and the kinetic parameters were determined by the *Solver* program (Excel® software), using the

method of least squares as an optimization (between experimental values and values calculated by the kinetic model).

Estimation of energy production

To estimate the energy production (E_{CH_4}) and the reactor design on an industrial scale ($(V_R)_{IND3}$ (L), a method proposed by Albanez et al. (2016) was used. Vinasse production data were obtained from an ethanol plant located in São Paulo, Brazil. The removed volumetric organic load ($RVOL_{MO}$ – $gCOD.L^{-1}.d^{-1}$) and yield of methane generated per consumed load (Y_{MO} – $mol_{CH_4}.gCOD^{-1}$) were estimated as described in Almeida et al. (2017).

This estimation approach, using industrial data, was carried out only with the objective to demonstrate a preliminary application of the results obtained in this work and to provide information for full-scale use of the investigated technological configuration (AnSBBR). It should be mentioned that detailed studies concerning operational aspects have to be considered to give a precise prediction of the scale-up system. Hewitt et al. (2010) reported a reduction of efficiency in large scale systems for aerobic batch reactors and discussed the importance of impeller type, rotor speed and agitation mode to ensure mass transfer and cell integrity. The dependence between mass transfer, impeller type and consumed power per volume (kW/m^3) were also studied by Michelan et al. (2009) using a bench scale anaerobic batch reactor with granular biomass (ASBR), and Novaes et al. (2010) using a pilot scale anaerobic batch reactor with granular biomass (ASBR) and immobilized biomass (AnSBBR).

The methane production ($Prod_{CH_4} = n_{CH_4}$) and combustion energy (ΔH_{C-CH_4}) were used to estimate the generated energy (E_{CH_4} – $MWh.month^{-1}$) by means of the daily molar production of methane ($Prod_{CH_4}$ – $mol_{CH_4}.d^{-1}$) and the methane enthalpy of combustion ($\Delta H_{C-CH_4} = 803 kJ.mol^{-1}$ – Perry, 1997) in Equations (36) and (37).

$$Prod_{CH_4} = (V_R)_{IND3} \cdot RVOL_{MO} \cdot Y_{MO} \quad (36)$$

$$E_{CH_4} = Prod_{CH_4} \times \Delta H_{C-CH_4} \quad (37)$$

The energy yield per removed volumetric organic load (YE_{CH_4} – $kJ.kgCOD^{-1}$) was calculated by Equation (38) relating the energy power to the removed organic matter.

$$YE_{CH_4} = \frac{PrM \cdot \Delta H_{C-CH_4}}{RVOL_{MO}} \quad (38)$$

Through Equation (39) the amount saved per month was calculated by using the bioenergy generated

in the system, in which PWR_{AnSBBR} ($= E_{CH_4} - MW$) corresponds to the energy generated by the AnSBBR ($MW = 30.24 MWh.month^{-1}$), $\$_{un}$ is the fuel price ($US\$.m^{-3}$), ICP is the inferior calorific value and ρ is the density ($8,800 kcal.kg^{-1}$ and $0.74 kg.m^{-3}$ for natural gas; $10,100 kcal.kg^{-1}$ and $840 kg.m^{-3}$ for diesel oil, respectively – ANP, 2015).

$$\$_{recovered} = \frac{PWR_{AnSBBR} \cdot \$_{un}}{ICP \cdot \rho} \quad (39)$$

RESULTS AND DISCUSSION

Step I (adaptation of biomass)

Phase 1 comprised assays AT 1, AT 2, AT 3, AT 4 and AT 5, which were operated for seven days each. Table 1 shows the average values of the monitored variables. The stability of the system was confirmed by the results of bicarbonate alkalinity and acids accumulation in the reactor, which were $647 mgCaCO_3.L^{-1}$ and $504 mgHAc.L^{-1}$, respectively. In addition, it was observed that, with increasing organic load, alkalinity was generated/consumed and volatile acids accumulated. Removal efficiency as COD and Carbohydrate reached 81 % and 99 %, respectively. The results indicated that the biomass was adhered to the inert support and was adapted to the temperature of $55^\circ C$ and influent concentration of $5000 mgCOD.L^{-1}$ with molasses-based wastewater.

Phase 2 comprised assays M80/V20, M60/V40, M40/V60, M20/V80 and M0/V100, which were operated for at least seven days each (total of 40 days). The purpose of this phase was to change the substrate used in the reactor start-up, i.e., molasses to vinasse. As shown in Table 1, the total volatile acids concentration in the influent increased (448 to $800 mgHAc.L^{-1}$) when vinasse was added to the wastewater composition, which led to a drop in the effluent pH to 6.9. During the first two conditions total volatile acids oscillated due to an imbalance in the microbial community present in the medium. In the following tests the pH remained near 8.0. Conversely, this condition led to a decrease of approximately 82 % in the TVA concentration in the effluent (504 to $62 mgHAc.L^{-1}$) and a nearly 150% increase in the buffer capacity (647 to $1617 mgCaCO_3.L^{-1}$), proving that vinasse improved system stability, despite its acidity. Consumption of intermediate acids increased during the cycle, mainly acetic and propionic acids (351 to $10 mg.L^{-1}$ and 105 to $0 mg.L^{-1}$, respectively). Organic matter removal as COD also improved when vinasse was added, achieving an efficiency of 87 % at condition M0/V100. On the other hand, the carbohydrate removal efficiency was lower (99 to 94 %) because of the poor biodegradability of vinasse.

Table 1. Parameters monitored in Step I.

Parameter	Phase 1					Phase 2				
	AT 1	AT 2	AT 3	AT 4	AT 5	M80/V20	M60/V40	M40/V60	M20/V80	M0/V100
Molasses (%)	100	100	100	100	100	80	60	40	20	0
Vinasse (%)	0	0	0	0	0	20	40	60	80	100
T (°C)	35	40	45	50	55	55	55	55	55	55
C _{MO-inf} (mgCOD.L ⁻¹)	1121	2193	2959	3938	5197	5069	5345	5410	5060	4810
C _{MOF} (mgCOD.L ⁻¹)	267	431	565	781	1001	943	661	456	630	632
ε _{MOF} (%)	76	81	81	80	81	81	88	92	88	87
C _{CT-inf} (mgCarbohydrate.L ⁻¹)	665	1314	1565	2329	2957	2628	1990	1544	1669	976
C _{CF} (mgCarbohydrate.L ⁻¹)	14	16	25	31	33	48	39	44	54	62
ε _{CF} (%)	98	99	98	99	99	98	98	97	97	94
pH _{inf} (u)	8.3	8.3	8.3	8.2	8.2	7.8	7.6	7.5	7.0	6.9
pH _{eff} (u)	7.4	7.4	7.6	7.7	7.7	7.8	7.9	8.0	8.0	8.0
TVA _{inf} (mgHAc.L ⁻¹)	39	72	94	143	151	448	745	852	815	800
TVA _{eff} (mgHAc.L ⁻¹)	140	182	225	339	504	421	106	59	62	76
BA _{inf} (mgCaCO ₃ .L ⁻¹)	346	692	612	688	958	665	416	435	199	201
BA _{eff} (mgCaCO ₃ .L ⁻¹)	393	630	704	1027	647	858	1350	1603	1617	1605
TS _{inf} (mg.L ⁻¹)	—	—	—	—	6307	5560	5848	6138	5773	5738
TS _{eff} (mg.L ⁻¹)	—	—	—	—	3178	2485	2595	2761	3128	3240
TSS _{inf} (mg.L ⁻¹)	—	—	—	—	93	97	148	211	169	171
TSS _{eff} (mg.L ⁻¹)	—	—	—	—	113	106	130	83	123	89
VSS _{inf} (mg.L ⁻¹)	—	—	—	—	54	60	125	163	107	121
VSS _{eff} (mg.L ⁻¹)	—	—	—	—	87	89	104	63	74	62

As shown in Table 2, addition of vinasse improved the methane fraction (73 %) in the generated biogas. Up to condition M20/V80 an improvement can be seen in the molar productivity of methane (87 molCH₄.m⁻³.d⁻¹) and the yield of methane generated by consumed organic matter (14.5 mmolCH₄.gCOD⁻¹ / 324 NmLCH₄.gCOD⁻¹). In contrast, the condition M0/V100 (vinasse-based wastewater) presented a slight decrease in

the yield attributed to the vinasse composition and the biomass acclimatization to the substrate (65 molCH₄.m⁻³.d⁻¹ and 11.7 mmolCH₄.gCOD⁻¹ / 263 NmLCH₄.gCOD⁻¹, respectively). Nonetheless, these results indicate that the reactor was stable and had a buffering capacity that was able to prevent accumulation of acids. Therefore, at the end of this step the biomass was adapted to treat the vinasse-based wastewater at 55 °C.

Table 2. Performance indicators on Step I.

Indicator	Phase 1					Phase 2				
	AT 1	AT 2	AT 3	AT 4	AT 5	M80/V20	M60/V40	M40/V60	M20/V80	M0/V100
M _{TVS} (gTVS)	51.1	51.1	51.1	51.1	51.1	64.3	64.3	64.3	64.3	64.3
C _X (gTVS.L ⁻¹)	21.5	21.5	21.5	21.5	21.3	26.6	27.2	27.1	27.1	27.1
AVOL _{MO} (gCOD.L ⁻¹ .d ⁻¹)	1.0	1.0	1.0	1.0	7.05	7.04	7.22	7.35	6.90	6.52
RVOL _{MO} (gCOD.L ⁻¹ .d ⁻¹)	1.5	3.0	4.0	5.3	5.6	5.6	6.1	6.6	6.0	5.5
AVOL _C (gCarbohydrate.L ⁻¹ .d ⁻¹)	1.1	2.4	3.2	4.2	4.1	3.7	2.7	2.1	2.3	1.3
RVOL _C (gCarbohydrate.L ⁻¹ .d ⁻¹)	0.9	0.9	2.1	3.2	4.0	3.6	2.6	2.0	2.2	1.2
V _G (NmL.cycle ⁻¹)	—	—	—	—	2278	2142	2069	2141	2119	1701
V _{CH4} (NmLCH ₄ .cycle ⁻¹)	—	—	—	—	1424	1406	1409	1519	1534	1152
n _{CH4} (molCH ₄ .d ⁻¹)	—	—	—	—	0.19	0.19	0.19	0.20	0.21	0.15
MPr (molCH ₄ .m ⁻³ .d ⁻¹)	—	—	—	—	79	78	80	86	87	65
SMP _r (molCH ₄ .kgTVS ⁻¹ .d ⁻¹)	—	—	—	—	3.7	2.9	2.9	3.2	3.2	2.4
VPr (NmLCH ₄ .L ⁻¹ .d ⁻¹)	—	—	—	—	1779	1743	1788	1918	1938	1458
SVPr (NmLCH ₄ .gTVS ⁻¹ .d ⁻¹)	—	—	—	—	84	66	66	71	72	54
Y _{MO} (mmolCH ₄ .gCOD ⁻¹)	—	—	—	—	14.2	13.9	13.1	13.0	14.5	11.7
Y _{MO} (NmLCH ₄ .gCOD ⁻¹)	—	—	—	—	314	313	292	290	324	263
X _{CH4} (%)	—	—	—	—	63	66	68	71	72	73
X _{CO2} (%)	—	—	—	—	36	34	32	29	28	27
HAc (mmol.L ⁻¹)	—	—	—	—	351	196	12	5	9	10
HPr (mmol.L ⁻¹)	—	—	—	—	105	188	11	0	0	0
HBu (mmol.L ⁻¹)	—	—	—	—	33	33	0	0	0	0
HVa (mmol.L ⁻¹)	—	—	—	—	39	35	0	0	0	0

Step II (optimization of applied organic load)

Step II comprised assays B5, B6, B7, B8, B9, B10, B12, B14, B17 and B20. The reactor was operated for 110 days in batch mode and the vinasse-based wastewater was supplemented with bicarbonate and urea. As shown in Table 3, the increase in influent concentration (with a consequent drop in the influent pH from 8.2 to 5.8) had no influence on the effluent pH value, which ranged from 8.0 at condition B5 (5000 mgCOD.L⁻¹) to 8.5 at B20 (20000 mgCOD.L⁻¹). In fact, even when bicarbonate supplementation was reduced to half in the wastewater from condition B9 (9000 mgCOD.L⁻¹), the pH exceeded 8.3, i.e., beyond the 6.5 to 8.0 optimum range for thermophilic methanogenic microorganisms (Paulo et al., 2003); behavior similar to that reported in the investigation of Song et al. (2004) in which the pH remained above 8.0 for thermophilic reactors. Even when TVA accumulation increased with increasing vinasse concentration in the effluent, the system showed improvement in buffer capacity. Hence, in case of an imbalance in TVA concentration the system still remains stable.

Table 3 shows that the organic matter concentration in the form of COD and carbohydrate in the effluent increased as organic load increased. The removal efficiency of organic matter as COD exceeded 80 % (ϵ_{CMO}) and as carbohydrate was 93 % (ϵ_{C}) for the filtered samples at all experimental conditions. The difference between removal efficiencies of COD and carbohydrate can be justified by the presence of poorly biodegradable compounds in the vinasse composition. It should be mentioned that, after the condition changed, both volatile acids consumption and the

efficiency of organic matter removal decreased. The increase in solid concentration in the reactor was due to the higher volume of vinasse in the wastewater composition. Despite this fact, there was no large variation in the organic fraction of the solids.

Regarding the performance indicators (Table 4), the biogas volumetric production followed the increase in applied organic load, the maximum volume of biogas (6828 NmL.cycle⁻¹), volume of methane (5196 NmLCH₄.cycle⁻¹) and molar yield (305 mmolCH₄.m⁻³.d⁻¹) was achieved at condition B20 (20000 mgCOD.L⁻¹). The mole fraction of methane in the biogas composition was slightly affected during the organic volumetric load increase, ranging from 73 % at B5 (5000 mgCOD.L⁻¹) to 78 % at B20 (20000 mgCOD.L⁻¹). The yield between generated methane and consumed organic matter was near the theoretical one (15.6 mmolCH₄.gCOD⁻¹ / 350 NmLCH₄.gCOD⁻¹) of condition B8 (8000 mgCOD.L⁻¹). As far as the intermediate compounds are concerned, Table 4 shows there was a predominance of acetic acid, followed by propionic acid at lower concentration. These performance indicators demonstrate the stability of the system and the equilibrium and interaction between the thermophilic microbiological community present in the biomass.

The relationship between the removed and applied organic volumetric loads (Figure 2) indicated that organic matter removal efficiency as COD during Stage II was 79 %. The linear behavior indicated that there was no inhibition by the organic load or imbalance in the produced/consumed intermediate compounds. The methane productivity and the removed volumetric

Table 3. Monitored parameters in Steps II and III.

Parameter	B5	B6	B7	B8	B9	B10	B12	B14	B17	B20	BA20
C _{MO-inf} (mgCOD.L ⁻¹)	4810	6196	7181	8259	8947	10345	12270	13632	17068	20084	19440
C _{MOT-eff} (mgCOD.L ⁻¹)	718	890	1458	1273	1534	2204	2866	2792	4240	4103	4391
C _{MOF-eff} (mgCOD.L ⁻¹)	632	771	1285	1046	1260	1863	2235	2156	3386	2875	3435
ϵ_{MOT} (%)	85	86	80	85	83	79	77	80	75	79	77
ϵ_{MOF} (%)	87	88	82	87	86	82	82	84	80	85	82
C _{CT-inf} (mgCarbohydrate.L ⁻¹)	976	1173	1655	1277	1646	1703	2700	2623	3213	4504	3292
C _{CT-eff} (mgCarbohydrate.L ⁻¹)	67	88	110	98	95	116	217	203	310	380	313
C _{CF-eff} (mgCarbohydrate.L ⁻¹)	62	74	99	76	73	87	158	141	224	263	216
ϵ_{CT} (%)	93	92	93	92	94	93	92	92	91	91	90
ϵ_{CF} (%)	94	94	94	94	95	95	94	95	93	94	93
pH _{inf} (u)	6.9	6.9	6.9	5.9	5.2	5.8	6.4	6.8	5.7	5.5	4.9
pH _{eff} (u)	8.0	8.2	8.1	8.2	8.3	8.2	8.2	8.4	8.3	8.5	8.5
TVA _{inf} (mgHAc.L ⁻¹)	800	1023	1391	2000	2990	2138	2522	3269	4280	4884	5653
TVA _{eff} (mgHAc.L ⁻¹)	76	92	276	172	486	924	713	727	1284	1210	1526
BA _{inf} (mgCaCO ₃ .L ⁻¹)	201	288	57	0	0	66	51	25	0	0	0
BA _{eff} (mgCaCO ₃ .L ⁻¹)	1605	1878	2139	2386	2451	2284	3067	3964	3570	3991	3643
TS _{inf} (mg.L ⁻¹)	5738	6619	8713	8528	9925	9868	13886	15426	18964	22203	18683
TS _{eff} (mg.L ⁻¹)	3240	3979	4763	4516	4454	5631	7263	7992	9476	11038	9373
TSS _{inf} (mg.L ⁻¹)	171	257	268	334	120	216	372	354	655	510	807
TSS _{eff} (mg.L ⁻¹)	89	128	155	187	184	290	283	836	524	1125	792
SVS _{inf} (mg.L ⁻¹)	121	189	202	279	84	123	270	245	530	360	642
SVS _{eff} (mg.L ⁻¹)	62	101	119	148	159	204	211	626	367	836	605

Table 4. Parameter indicators in Step II and III.

Indicator	B5	B6	B7	B8	B9	B10	B12	B14	B17	B20	BA20
M _{TVS} (g)	64.3	75.7	75.7	75.7	75.7	75.7	86.2	86.2	106.1	120.6	121
C _X (gTVS.L ⁻¹)	27.1	31.9	32.7	32.2	31.6	31.6	37.0	37.5	46.1	52.8	53
C _X ³ .(gTVS.suporte ⁻¹)	1.0	1.8	1.8	1.8	1.8	1.8	1.9	1.9	2.6	2.9	2.9
AVOL _{MO} (gCOD.L ⁻¹ .d ⁻¹)	6.5	8.4	9.5	11.1	12.3	14.2	16.3	17.8	22.3	25.9	25
RVOL _{MO} (gCOD.L ⁻¹ .d ⁻¹)	5.5	7.2	7.5	9.4	10.2	11.2	12.5	14.1	16.7	20.6	19
AVOL _C (gCarbohydrate.L ⁻¹ .d ⁻¹)	1.3	1.6	2.2	1.7	2.3	2.3	3.6	3.4	4.2	5.8	4.3
RVOL _C (gCarbohydrate.L ⁻¹ .d ⁻¹)	1.2	1.5	2	1.6	2.1	2.2	3.3	3.2	3.8	5.3	3.8
V _{CH4} (NmLCH ₄ .cycle ⁻¹)	1152	1819	1827	2477	2891	3204	3268	3967	4422	5196	7391
n _{CH4} (molCH ₄ .d ⁻¹)	0.15	0.24	0.24	0.33	0.39	0.43	0.44	0.53	0.59	0.70	0.80
MPr (molCH ₄ .m ⁻³ .d ⁻¹)	65	102	106	141	162	179	188	231	257	305	352
SMPr (molCH ₄ .kgTVS ⁻¹ .d ⁻¹)	2.4	3.2	3.2	4.4	5.1	5.7	5.1	6.2	5.6	5.8	6.7
VPr (NmLCH ₄ .L ⁻¹ .d ⁻¹)	1458	2296	2365	3163	3622	4016	4211	5175	5765	6828	7888
SVPr (NmLCH ₄ .gTVS ⁻¹ .d ⁻¹)	54	72	72	98	115	127	114	138	125	129	149
Y _{MO} (mmolCH ₄ .gCOD ⁻¹)	11.7	14.2	14.0	15.1	15.9	16.1	15.1	16.3	15.4	14.8	18.1
Y _{MO} (NmLCH ₄ .gCOD ⁻¹)	263	319	315	336	355	359	337	365	345	331	407
X _{CH4} (%)	73	76	75	76	77	78	78	77	77	78	77
X _{CO2} (%)	27	24	25	24	23	22	22	23	23	22	23
HAc (mmol.L ⁻¹)	10	22	31	98	448	541	469	394	420	720	819
HPr (mmol.L ⁻¹)	0	0	0	72	87	99	119	131	152	257	280
HBu (mmol.L ⁻¹)	0	0	0	0	0	0	0	0	0	0	0

organic load (Figure 2) confirmed that methane productivity was directly related to the organic load increase, i.e., the higher the applied organic load, the greater the availability of organic matter to be converted to methane. The average value during Step II was 15.2 mmolCH₄.gCOD⁻¹ (341 NmLCH₄.gCOD⁻¹), near the theoretical value of 15.6 mmolCH₄.gCOD⁻¹ (350 NmLCH₄.gCOD⁻¹). The linear trend indicates that the applied organic load did not inhibit biogas generation.

The behavior of the reactor shown in Figure 2 allowed us to conclude that the maximum organic load that could be treated by this reactor configuration had not been reached, and reactor stability and performance in relation to the organic matter removal and methane production efficiency was not affected.

Table 5 shows the kinetic model parameters of the metabolic route regarding the change in the applied organic load. The specific parameters are represented by k'_{10M} for the acetoclastic route and k'_{11M} for the hydrogenotrophic route. It should be mentioned that the kinetic model adequately fitted the experimental data. In the hydrolysis and acidogenesis stage, condition B14 (14000 mgCOD.L⁻¹) presented higher

specific rate of organic matter consumption. Regarding the acidogenesis stage, the production rate of acetic acid via ethanol followed the increases in load. It should be mentioned that Condition B5 presented a higher value in the production of organic acids, which is justified by the value of volatile acids obtained during the monitoring. At the methanogenesis stage, methane production via the methanogenic acetoclastic route was predominant for all conditions and methane production via the hydrogenotrophic methanogenic route occurred at conditions B6 and B7.

Figure 3 shows the values obtained from the experimental data (markers) and those calculated by the kinetic model (line) referring to substrate consumption (a), production/consumption of intermediate compounds (b) and methane production (c) of the cycle for Condition B20, indicating the good fit between the data calculated by the model and the experimental data.

Step III (feed strategy)

Table 3 shows the results of the monitoring parameters of step III (BA20 – 20000 mgCOD.L⁻¹)

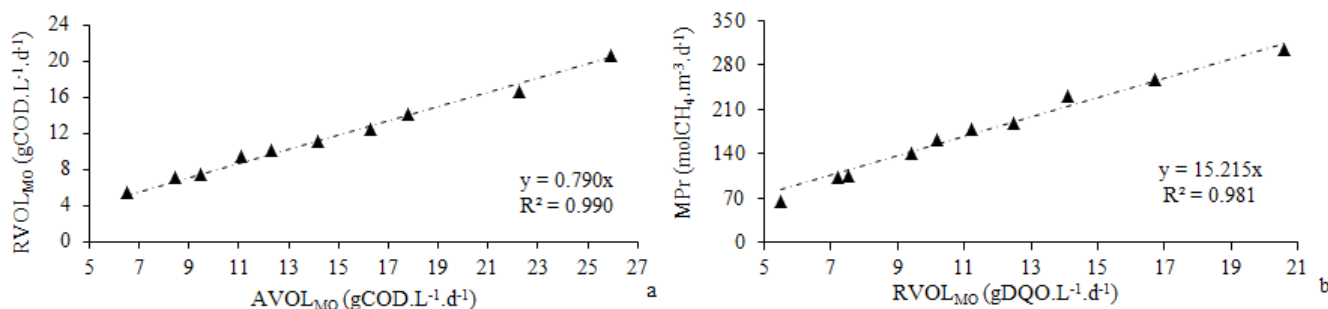
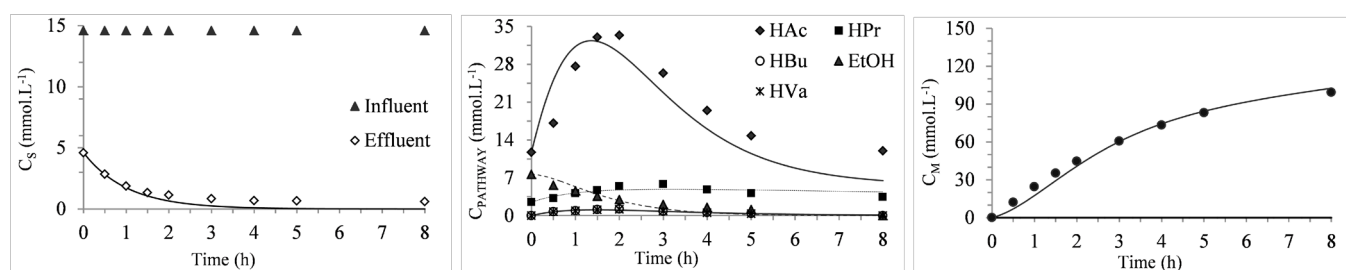
**Figure 2.** Efficiency of organic matter removal and methane molar productivity.

Table 5. Kinetic parameters for the conditions of Stages II and III.

Step	Kinetic Parameters	B5	B6	B7	B8	B9	B10	B12	B14	B17	B20	BA20
Hydrolysis		0.8	0.9	0.7	0.8	1.0	0.8	1.1	0.9	0.7	1.0	4.5
	k_s	0.8	0.7	0.9	0.9	0.0	1.1	1.6	0.0	1.4	0.0	41.5
Acidogenesis	k_{2HPR}	1.2	0.8	1.3	0.9	1.4	0.9	0.9	1.0	1.0	0.6	4.8
	k_{3HBU}	1.3	0.4	0.8	0.4	0.7	0.3	0.1	0.6	0.5	0.4	2.3
	k_{4HVA}	0.6	0.2	0.3	0.6	0.8	0.3	0.4	0.6	0.7	0.4	1.1
	k_{5ETOH}	0.1	0.0	0.0	0.0	0.8	0.0	0.2	1.2	2.1	1.2	1.5
	k_{HPR}	0.0	0.0	0.2	0.4	1.5	0.6	1.4	1.8	2.2	1.2	8.5
	k_{9HAC}	1.2	0.8	1.2	0.7	0.9	0.5	0.8	1.2	1.4	0.9	3.8
Acetogenesis	k_{EiOH}	2.0	1.4	1.8	2.4	3.4	2.7	3.5	5.2	5.4	5.5	21.4
	k_{6HPR}	0.7	0.4	0.6	0.3	0.1	0.1	0.1	0.1	0.1	0.0	0.1
	k_{7HPR}	1.5	0.2	0.4	1.6	0.0	0.1	0.3	0.0	0.0	0.0	0.0
	k_{7HBU}	7.0	1.0	3.0	1.4	0.6	0.2	1.3	1.3	4.5	0.4	0.6
	k_{8HBU}	4.2	0.1	1.0	1.1	0.0	0.1	1.3	0.8	4.6	0.0	0.0
	k_{8HVA}	1.0	0.4	0.6	0.7	0.7	0.1	0.4	0.6	0.7	0.5	0.3
Methanogenesis	K_{10M}	1.3	1.6	1.4	0.8	0.7	0.6	0.8	0.8	0.9	0.7	0.0
	K_{11M}	0.0	0.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9

**Figure 3.** Profiles of organic matter, intermediate compounds and methane at Condition B20.

when the reactor was operated for 25 days in fed-batch mode and the vinasse-based wastewater was supplemented with bicarbonate and urea. The pH was close to 8.5, when consumption of bicarbonate alkalinity and accumulation of total volatile acids were higher, due to the fact that production/consumption occurred concurrently with the input of the organic matter, since the feeding time was half the cycle length. Despite this fact, the imposed feeding strategy proved to be able to neutralize the acids produced by the system.

The organic matter removal efficiency (Table 3) as COD was 82 % and as carbohydrate exceeded 93 % for filtered samples. In relation to the organic fraction of the solids, there was little variation using this feeding strategy. Regarding the performance of the reactor the results indicated proper functioning of the reactor to treat effluents with high organic matter concentration.

Table 4 presents the results regarding the performance indicators; the fed-batch mode strategy showed superior performance in relation to methane production, in which the molar flow was $0.80 \text{ molCH}_4 \cdot \text{d}^{-1}$, molar productivity of methane was $352 \text{ molCH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and the volumetric productivity $7888 \text{ NmLCH}_4 \cdot \text{L}^{-1} \cdot \text{d}^{-1}$. In addition, the feed strategy did not affect the biogas composition in terms of methane, which remained near 77 %. The molar yield of methane produced by the removed organic matter

was $18.1 \text{ mmolCH}_4 \cdot \text{gCOD}^{-1}$ ($407 \text{ NmLCH}_4 \cdot \text{gCOD}^{-1}$). This value beyond the theoretical yield was justified by the measurement of organic matter as COD, which contemplates compounds formed during the metabolism (extracellular polymers – mainly produced in the fed-batch assay) that were dissolved due to the thermophilic condition. Hence, at the end of a cycle the effluent containing these compounds increases the COD value and the molar yield of methane produced by the removed organic matter becomes overestimated. However, despite this fact, the results indicate that this feeding strategy had a positive effect on the microorganisms metabolism and stimulated the production of biogas.

Analysis of the kinetic parameters of the metabolic route (Table 4) allowed us to identify that the fed batch condition presented a higher specific rate of organic matter consumption (hydrolysis and acidogenesis), as well as of acetic acid production. In the acetogenesis stage, the priority route for acetic acid production resulted in higher propionic acid and ethanol consumption. The higher organic acids production contributed to acid accumulation in the system. In the methanogenesis stage methane formation was mainly due to the hydrogenotrophic route.

Figure 4 exhibits the values obtained from the experimental data (symbols) and those calculated by the kinetic model (line) referring to the substrate

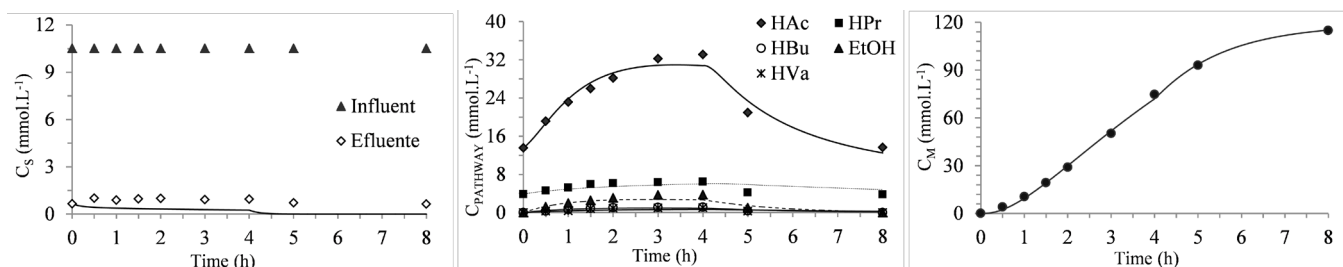


Figure 4. Profiles of organic matter, intermediate compounds and methane in Condition BA20.

consumption (a), production and consumption of intermediate compounds (b) and methane production (c) along the cycle for condition BA20, showing that the data calculated by the kinetic model fitted the experimental data.

Estimation of energy production

The estimation of energy production (Table 6) was performed considering the condition which presented the best results, i.e., Condition BA20 (fed batch – 20000 mgCOD.L⁻¹). The vinasse production and harvest period were 1.962×10^6 m³ and 253 days, respectively. An industrial reactor volume of 20,300 m³ was estimated to treat vinasse, and four AnSBBR reactors of 5084 m³ each, working in parallel, were proposed to render the industrial operation feasible.

The estimated power recovered in this process was 30.3 MW, i.e., 2.2×10^4 MWh per month, equivalent to the consumption of 134×10^3 habitants, considering a Brazilian average energy consumption per capita of 163 kWh.month⁻¹ in the first semester of 2017 (ANEEL, 2017). For an alcohol plant using natural gas or diesel oil as fuel in the process, the bioenergy produced in the AnSBBR corresponds to a saving of US\$ 2,167,000 or US\$ 2,234,000 per month, respectively. Values for natural gas and diesel oil were considered according to ANP (2017), US\$ 0.752 m⁻³ and US\$ 1,011 m⁻³, respectively, and a dollar rate of US\$ 1.000/R\$ 3.213 (BCB, 2017).

Comparing AnSBBR configurations treating vinasse, Albanez et al. (2016) estimated an energy production of 1.3×10^4 MWh per month, using biogas from an AnSBBR reactor at mesophilic conditions, with an influent concentration of 5000

mgCOD.L⁻¹, whereas the estimated energy generation at thermophilic conditions for the same influent concentration in our investigation was higher (1.6×10^4 MWh per month). Data reported by Almeida *et al.* (2017) allowed us to estimate an energy production from the biogas generated in an AnSBBR reactor at mesophilic condition of 1.8×10^4 MWh per month for the influent concentration of 10000 mgCOD.L⁻¹. The estimated energy generation for the same influent concentration in our investigation was 2.1×10^4 MWh per month. On analyzing the estimated energy generated from the biogas produced in an AnSBBR reactor treating vinasse, the thermophilic configuration proved to be more efficient. It should be mentioned that technological possibilities for harvesting biogas energy include boilers and turbines, which should be studied to estimate efficiency and economical aspects.

It should be mentioned that the estimation of the energy production and the reactor volume using industrial data was carried out only with the objective to demonstrate a preliminary application of the results obtained in this work and to provide information for full-scale use of the investigated technological configuration (AnSBBR).

Comparative analysis with the literature

A comparison of the results of this study with investigations found in the literature which dealt with reactors that treat vinasse is shown in Table 7. In the current study, an AnSBBR reactor operated at thermophilic conditions to treat AVOL of 25.9 gCOD.d⁻¹.L⁻¹ achieved organic matter removal efficiency as COD of 85 % and methane yield produced per removed organic matter of 330 NmLCH₄.gCOD⁻¹. The

Table 6. Estimated energy production.

Parameters	B5 (this work)	B10 (this work)	B20 (this work)	BA20 (this work)	Albanez et al. (2016)	Almeida et al. (2017)
Temperature (°C)	55	55	55	55	30	30
C _{MOaI} (mgCOD.L ⁻¹)	4810	10345	20084	19440	5000	10000
C _{MOTeff} (mgCOD.L ⁻¹)	720	2204	4103	4391	977	261
RVOL (gCOD.L ⁻¹ .d ⁻¹)	5.5	11.2	20.6	19.4	4.58	9.8
n _{CH4} (molCH ₄ .d ⁻¹)	2.4×10^6	3.2×10^6	2.7×10^6	3.3×10^6	1.9×10^6	2.7×10^6
Y _{MO} (mmolCH ₄ .gCOD ⁻¹)	11.7	16.1	14.8	18.1	9.5	12.6
Y _{MO} (NmLCH ₄ .gCOD ⁻¹)	263	359	331	407	213	282
Y _{EMO} (kJ.kgCOD ⁻¹)	9.4×10^3	1.3×10^4	1.2×10^4	1.5×10^4	7.6×10^3	1.0×10^4
Energy – E _{CH4} (MWh.month ⁻¹)	1.6×10^4	2.1×10^4	1.8×10^4	2.2×10^4	1.3×10^4	1.8×10^4

Table 7. Results of studies reported in the literature.

Reactor	t_c^1/HHT^2 (h)	T (°C)	AVOL (gCOD.L ⁻¹ .d ⁻¹)	ϵ_{MOF} (%)	Volumetric Y_{MO} (NmLCH ₄ .gCOD ⁻¹)	VPr (NmLCH ₄ .L ⁻¹ .d ⁻¹)
AnSBBR ^a	0.2 ¹	55	26	78	331	6828
AnSBBR ^a	4.0 ¹	55	25	77	407	7888
UASB ^b	52.8 ²	55	25-30	72	400	-
UASB ^c	61.2 ²	55	24	45	290	-
UASB I ^d	34 ²	55	25	63	306	2232
UASB II ^d	23 ²	55	25	66	316	2947
AnSBBR ^e	0.2	55	5.0	69	-	-
AnSBBR ^f	0.2	45	10	46	160	785
AnSBBR ^f	0.2	30	5.2	97	283	1424
AnSBBR ^f	0.2	30	6.2	97	301	1815
AnSBBR ^f	0.2	30	10	97	281	2767
AnSBBR ^g	4.0	30	5.5	83	212	973

Notation: ^aThis work; ^bSilva et al. (1992); ^cHarada et al. (1996); ^dFerraz Junior (2016); ^eRibas et al. (2009); ^fAlmeida et al. (2017); ^gAlbanez et al. (2016).

results are superior to those reported by Harada et al. (1996), who operated a thermophilic UASB reactor, in which the COD removal efficiency was 40 % and the methane yield 290 NmLCH₄.gCOD⁻¹. The lower efficiency might be related to the difference in scale, the vinasse composition and the higher accumulation of propionate with increasing load. The results obtained in the current study were similar to those reported by Silva et al. (1992) who operated a pilot scale thermophilic UASB reactor and achieved 72 % removal efficiency of organic matter as COD and yield of 400 NmLCH₄.gCOD⁻¹.

Ferraz Junior et al. (2016) analyzed the behavior of a single-stage thermophilic UASB (I) reactor treating vinasse and a two-stage thermophilic UASB (II) reactor, treating effluent from an acidogenic reactor that also treated vinasse. The results obtained were inferior to those in this work for removal efficiency (I - 62.8 % and II - 65.7 %), volumetric yield of methane (I - 306 NmLCH₄.gCOD⁻¹ and II - 316 NmLCH₄.gCOD⁻¹) and methane fraction in the biogas (I - 58 % and II - 76 %).

Ribas et al. (2009) reported a 69 % organic matter removal efficiency as COD in an AnSBBR thermophilic reactor. When the AVOL was increased, higher instability was observed and supplementation of the bicarbonate was needed in the effluent. The lower yield and instability in the thermophilic reactor were attributed to the adaptation of the mesophilic sludge used, as well as to operational difficulties.

Albanez et al. (2016) treated vinasse at 30 °C in an AnSBBR similar to that used in the present investigation. The results reported were COD removal efficiency of 83 %, higher than that reached in the thermophilic condition. However, at the thermophilic condition studied in this work the yield of methane generated per organic matter consumed and the methane productivity were higher.

Almeida et al. (2017) analyzed an AnSBBR similar to the reactor used in the present work, operated at 30

°C and 45 °C. They achieved COD removal efficiency of 97 % at mesophilic condition, yield of methane produced per consumed organic load of 281 NmLCH₄.gCOD⁻¹, volumetric methane production of 2767 NmLCH₄.L⁻¹.d⁻¹ and methane fraction of 78 % in the biogas composition. At the thermophilic condition of 45 °C the reactor presented low yield of methane production (160 NmLCH₄.gCOD⁻¹), low methane volumetric production of 785 NmLCH₄.L⁻¹.d⁻¹ and organic matter removal efficiency of 46 %. The performance regarding removed organic matter and stability of the AnSBBR used in our study disagrees with that reported by Ribas et al. (2009) and Almeida et al. (2017), achieving better values in relation to the parameters mentioned by the authors. Therefore, the AnSBBR reactor containing immobilized biomass, operated under thermophilic condition, showed a feasible configuration for the treatment of vinasse. Furthermore, in the current literature, this reactor configuration has been increasingly used for the production of methane or hydrogen, as bioenergy can be produced from the treatment of various wastewaters, such as vinasse, whey, domestic sewage, effluent from biodiesel production and other industrial effluents (Bezerra et al., 2011; Oliveira et al., 2009; Rodrigues et al., 2011; Lovato et al., 2012; Albanez et al., 2016; Lima et al., 2016).

CONCLUSIONS

Methane production from vinasse wastewater by a thermophilic AnSBBR reactor was successfully achieved. Best results were obtained in the fed-batch mode at volumetric organic load of 25 gCOD.L⁻¹.d⁻¹, which led to average COD and carbohydrate removal of 82 % and 93 %, respectively, as well as methane productivity, yield and content in biogas of 352 molCH₄.m⁻³.d⁻¹, 18.1 mmolCH₄.gDQO⁻¹ (407 NmLCH₄.gDQO⁻¹) and 77 %, respectively. Fitting a kinetic model to the experimental data revealed

predominance of acetic acids and showed that methane production occurred primarily via the acetoclastic route.

The reactor showed its energy recovery potential in treating vinasse with high organic matter concentration. Using the results of the laboratory scale reactor, for a real scale treatment plant (vinasse production of $1.962 \times 10^6 \text{ m}^3$ and harvest period of 253 days) four 5084 m^3 AnSBBRs working in parallel (industrial reactor volume of 20,300 m^3) were estimated, resulting in an energy recovery of 2.2×10^4 MWh per month, equivalent to an energy consumption of 134×10^3 inhabitants in Brazil. In an alcohol plant, savings might reach US\$ 2,167,000 or US\$ 2,234,000 upon replacing natural gas fuel or diesel oil for the bioenergy produced in the AnSBBR, respectively.

The results obtained in this study proved that the digestion of vinasse in a thermophilic AnSBBR reactor is an efficient technological and environmental compliant alternative for methane production in the treatment of high organic load wastewater.

NOTATION

AnSBBR	Anaerobic Sequencing Batch Biofilm Reactor
ASBR	Anaerobic Sequencing Batch Reactor
AVOL	Applied volumetric organic load, expressed by COD or Carbohydrate ($\text{gCOD.L}^{-1}.\text{d}^{-1}$ or ($\text{gCarbohydrate.L}^{-1}.\text{d}^{-1}$)
BA	Bicarbonate alkalinity ($\text{mgCaCO}_3.\text{L}^{-1}$)
C_{Cinf}	Organic matter concentration in the influent ($\text{mgCarbohydrate.L}^{-1}$)
C_{CF}	Filtered organic matter concentration ($\text{mgCarbohydrate.L}^{-1}$)
COD	Chemical oxygen demand (mgCOD.L^{-1})
C_{MOinf}	Organic matter concentration in the influent (mgCOD.L^{-1})
C_{MOF}	Filtered organic matter concentration (mgCOD.L^{-1})
C_{X}	Mass of total volatile solids per volume of liquid medium (gTVS.L^{-1})
EtOH	Ethanol concentration (mg.L^{-1})
HAc	Acetic acid concentration (mg.L^{-1})
HBu	Butyric acid concentration (mg.L^{-1})
HPr	Propionic acid concentration (mg.L^{-1})
HVal	Valeric acid concentration (mg.L^{-1})
MPr	Mol of methane per day and per reactor volume ($\text{molCH}_4.\text{m}^{-3}.\text{d}^{-1}$)
M_{TVS}	Mass of total volatile solids into the reactor (gTVS)
n_{CH_4}	Moles of methane produced ($\text{molCH}_4.\text{d}^{-1}$)
pH	Hydrogen ion potential
RVOL	Removal volumetric organic load, expressed by COD or Carbohydrate ($\text{gCOD.L}^{-1}.\text{d}^{-1}$ or ($\text{gCarbohydrate.L}^{-1}.\text{d}^{-1}$)

SMPr	Moles of methane per day and per reactor TVS ($\text{molCH}_4.\text{kgTVS}^{-1}.\text{d}^{-1}$)
SVPr	Volume of methane per day and per reactor TVS ($\text{NmLCH}_4.\text{gTVS}^{-1}.\text{d}^{-1}$)
TS	Total solids (mgTS.L^{-1})
TSS	Total suspended solids (mgTSS.L^{-1})
TVA	Total volatile acids (mgHAc.L^{-1})
VSS	Volatile suspended solids (mgVSS.L^{-1})
V_{G}	Volume of biogas at STP (NmL.cycle^{-1})
V_{CH_4}	Volume of methane at STP ($\text{NmLCH}_4.\text{cycle}^{-1}$)
V_{R}	Volume of liquid into the reactor (L)
V_{G}	Volume of biogas at STP (NmL.cycle^{-1})
VPPr	Volume of methane per day and per reactor volume ($\text{NmLCH}_4.\text{L}^{-1}.\text{d}^{-1}$)
X_{CH_4}	Percentage of methane in biogas (%)
X_{CO_2}	Percentage of carbonic gas in biogas (%)
Y_{MO}	Molar yield of methane from consumed organic matter ($\text{mmolCH}_4.\text{gCOD}^{-1}$)
Y_{MO}	Volumetric yield of methane from consumed organic matter ($\text{NmLCH}_4.\text{gCOD}^{-1}$)
ϵ_{MOF}	Removal efficiency of filtered organic matter (%)

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