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Pre-treatment of filter cake for anaerobic digestion in sugarcane biorefineries: Assessment of batch versus semi-continuous experiments

Leandro Janke[a*], Sören Weinrich[a], Athaydes F. Leite[b], Heike Sträuber[b], Marcell Nikolausz[b], Michael Nelles[a,c], Walter Stinner[a]

[a] Department of Biochemical Conversion, Deutsches Biomasseforschungszentrum gemeinnützige GmbH, Torgauer Straße 116, 04347 Leipzig, Germany

[b] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

[c] Faculty of Agricultural and Environmental Sciences, Chair of Waste Management, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany

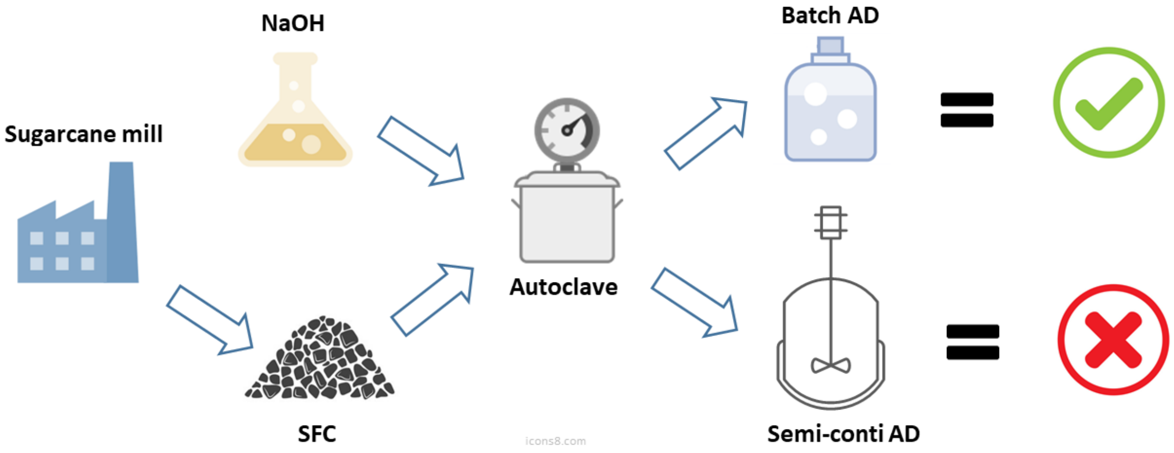
*Corresponding author

Tel.: +49 (0) 341 2434-793, Fax: +49 (0) 341 2434-133

Email: leandro.janke@dbfz.de

Graphical Abstract

Graphical abstract



Highlights:

- Different pre-treatment methods were assessed on AD of SFC
- During BMP tests autoclave with NaOH improved ($p < 0.05$) methane potential
- Methane yield was not improved ($p > 0.05$) during semi-continuous experiments
- Microbial community adaptation might have influenced semi-continuous experiments

Abstract

Anaerobic digestion (AD) of sugarcane filter cake (SFC) was investigated by comparing the performance of pre-treatment methods in biochemical methane potential (BMP) tests and semi-continuous experiments. For that, SFC was pre-treated by autoclaving the substrate alone or with sodium hydroxide (NaOH). Experimental data from BMP tests were fitted to a kinetics model and further used for simulating the AD process in a continuous stirred-tank reactor (CSTR). BMP tests showed differences ($p < 0.05$) in total methane potential (S_{BMP}), which have affected methane yields during simulation in a CSTR. Untreated produced 185 mL CH_4 g_{VS}^{-1}, autoclaved pre-treatment 174 mL CH_4 g_{VS}^{-1} and autoclaved with NaOH pre-treatment 222 mL CH_4 g_{VS}^{-1}. Interestingly, such higher performance of autoclaved with NaOH pre-treatment was only observed at earlier stages during semi-continuous feeding experiment. At steady-state no significant differences ($p > 0.05$) in terms of methane yield were observed among the reactors (average of 224 mL CH_4 g_{VS}^{-1}). These results demonstrate that the benefits of pre-treatment could only be observed in BMP tests, which is likely explained by a better adaptation of the microbial community to the substrate during long term semi-continuous experiment, making SFC pre-treatment ineffective in a single-stage CSTR and under this feeding regime.

Keywords: Biomass conversion; degradation kinetics; feeding regime; methane potential.

1. Introduction

The anaerobic digestion (AD) process has been proven to be an alternative biomass conversion pathway to diversify the product portfolio of sugarcane biorefineries by recovering methane-rich biogas, promoting sustainable waste management practices and reducing greenhouse gas (GHG) emissions [1].

Sugarcane filter cake (SFC) is a solid waste generated during the clarification (physical–chemical process) of sugarcane juice before been used for sugar and first generation bioethanol production. SFC is mainly composed of water, inorganic soil particles, residual sugars and small pieces of sugarcane bagasse, which are often intentionally added to improve the permeability during the recovery of sucrose at the rotary vacuum-drum filter [2]. In contrast to the other solid sugarcane waste, namely straw and bagasse, SFC is not a suitable material to be used as fuel in conventional co-generation systems due to its high moisture and mineral contents [3]. The AD of SFC would allow the recovery of a valuable fuel (methane) to be used in different applications, and at the same time maintaining the mineral content (especially phosphorus) of the digestate allowing its proper use as fertilizer on the sugarcane fields [4,5].

Different studies reported the recalcitrance of SFC in AD processes due to its lignocellulosic structure which prevents the action of microorganisms and enzymes [6,7]. Such drawback can potentially result in lower methane yields and longer hydraulic retention times (HRT) directly affecting the profitability of biogas plants in large-scale applications [8].

However, most part of these studies have been limited to assess the effects of biomass pre-treatment on biochemical methane potential (BMP) tests under batch conditions [6–9]. A previous study from our group on the AD of sugarcane straw co-digested with SFC showed that BMP tests did not provide a good estimation of the semi-continuous feeding process in a continuous stirred-tank reactor (CSTR), possibly due to the different source, activity and inoculum adaptation as well as feeding mode [10]. Therefore, only by applying a feeding regime used in large-scale applications (semi-continuous) it is possible to have a proper understanding of the benefits of biomass pre-treatment in terms of enhanced methane yields, faster conversion kinetics and improved mixing in CSTRs.

In addition, in case chemical reagents, such as sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), aqueous ammonia (NH_4OH), are used to catalyze the pre-treatment, inhibitors to the AD process (e.g. Na^+, H_2S and NH_3) could be released resulting in process failure [11]. Thus, reinforcing the necessity of assessing the effects of biomass pre-treatment during a feeding regime used in large-scale, since in BMP tests a higher share of inoculum in relation to the substrate is used, which dilutes inhibitors and mislead proper conclusions [12].

In this study, the effects of SFC autoclaving and autoclaving with NaOH were investigated in batch and semi-continuous feeding mode to assess whether these pre-treatment techniques could provide the same benefits in terms of process stability, degradation kinetics and methane yield. Such approach can provide better understanding on how these pre-treatment techniques occur in practice, resulting in important inputs for process designing leading to a more efficient resource utilization.

2. Material and methods

2.1. Substrate and inoculum

SFC was obtained from a distillery plant in the state of Goiás (Brazil) during the 2014/2015 season, transported to Germany in sealed plastic containers and stored at 4

°C until its use. A German large-scale biogas plant that uses maize silage and cattle manure as substrate provided fresh digestate, which was used as inoculum for the BMP tests and the semi-continuous experiment [13,14].

2.2. Substrate pre-treatment

SFC pre-treatment was carried out in 500 mL glass flasks with an alkaline reagent concentration of 6 g NaOH/100 g SFC based on fresh matter (FM). The substrate total solid (TS) content was 83 g L^{-1}. SFC and NaOH solution was manually mixed and autoclaved for 30 min at 121 °C and 1 bar overpressure in a semi-automatic benchtop autoclave 2540 ML (Tuttnauer, Netherlands). After pre-treatment, SFC was neutralized with nitric acid (HNO_3) and stored at 4 °C until its use.

2.3. Biochemical methane potential tests

The BMP of untreated, autoclaved and autoclaved with NaOH pre-treated SFC was determined according to VDI (2016) using an Automatic Methane Potential Test System II (Bioprocess Control, Sweden) at mesophilic temperature (38 ± 1 °C) for 30 days. Prior to the BMP tests, the inoculum was degassed for 7 days at 38 °C to reduce non-specific biogas production. To prevent inhibition, the ratio of substrate/inoculum ($g_{VS}\ g_{VS}^{-1}$) was set to 0.44 ± 0.09 (i.e. around 2.3 times higher amount of inoculum than substrate based on volatile solids (VS)). The pH value in each batch reactor was measured before and after the BMP tests.

2.4. Semi-continuous experiment

Three lab-scale CSTRs, each with 5 L total volume (3 L working volume), were used for this experiment. The reactors were continuously stirred (100 rpm) using a central stirrer with vertical shaped blades to reduce the formation of floating layers. The operation temperature was kept at mesophilic conditions (38 ± 1 °C) by recirculating hot water through the double-walled reactors.

The experiment was carried-out over 90 consecutive days in two phases (start-up and constant operation) until reaching a technical steady-state after completing 3 turnovers based on the HRT [12]. For comparison, all CSTRs were fed with the same feeding frequency (once per day), organic loading rate (OLR), and HRT, only differing in substrate pre-treatment (untreated, autoclaved and autoclaved with NaOH pre-treated). For the start-up phase (days 0-15), the OLR was gradually increased from 2.1 to 3.2 g_{VS} L^{-1} d^{-1} while the HRT was decreased from 38.5 to 25 d. After this period, the OLR and HRT were kept stable until the end of the experiment at 3.2 g_{VS} L^{-1} d^{-1} and 25 d, respectively. To provide sufficient alkalinity and nitrogen as a nutrient 2 g of urea ($(NH_2)_2CO$) per liter of feeding was daily supplemented to all reactors [15]. In addition, to balance the missing macro (C:S ratio) and micronutrients of the substrate, a nutrient solution based on 0.6 g S, 0.9 g Mn, 4.9 mg Co, 20.9 mg Cu, 16 mg Mo, 12 mg Ni, 5 mg W, 285 mg Zn and 2 mg Se per kg of TS was daily supplemented to the reactors according to [16–18]. Detailed information about the different feeding rates, OLR and HRT in each phase of the experiment is listed in **Table 1**.

[insert Table 1 here]

2.5. Analytical methods

TS and VS and of substrate and digestate were determined by drying the samples for 24 h at 105 °C in a drying oven (Binder, Germany) and further reducing the organic content to ashes for 2 h at 550 °C in a high temperature oven (Carbolite, UK). The TS content of SFC was corrected to account for losses of volatile compounds during oven drying according to Weißbach & Strubelt [19]. The solubilized COD (S_{COD}) of centrifuged SFC after pre-treatment was analyzed using a LCK 014 COD kit (Hach-Lange, Germany) according to the manufacturer's protocol. Crude protein, crude lipids, crude fiber and nitrogen-free extractives of SFC were determined according to Weender method [20]. To determine the macro elements composition of SFC (C, H, N, S), about 30 mg of the substrates and 30 mg of WO_3 were weighted and pressed in an aluminum foil for subsequent burning at 1150 °C catalytically with oxygen. The combustion gases were directed through a reduction tube where NOx was reduced to N_2. The remaining gases (CO_2, H_2O, SO_2) passed through three different adsorption columns and were detected with a thermal conductivity detector (C, H, N) and an infrared spectroscopy detector (S) by using a Vario Macro Cube (Elementar, Germany) [21]. To determine the trace elements composition, dried samples were pre-treated with a mixture of $HNO_3/H_2O_2/HF$ and latter neutralized with H_3BO_3, and the resulting clear solution was analyzed by inductively coupled plasma atomic spectrometry – ICP-OES (ThermoFisher iCAP6200) [22].

The daily biogas production in the CSTRs was measured by a drum-type gas meter TG 05 (Ritter, Germany), and corrected to standard temperature and pressure conditions (273.15 K and 101.325 kPa). The composition of the biogas (CH_4, CO_2, O_2 and H_2S) was measured once a week at the headspace of the CSTRs by using a GA2000 Landfill Gas Analyzer (Geotechnical Instruments, UK). The specific methane production (SMP) is presented in milliliters per g of VS or COD (mL g_{VS}^{-1} or mL g_{COD}^{-1}) and the volumetric methane production (VMP) calculated in liters of methane per liter of reactor per day (L L^{-1} d^{-1}).

Five days per week, the pH value of fresh digestate of the CSTRs was measured immediately after sampling (before feeding) with a pH-electrode Sentix 41 (WTW, Germany). Once a week, digestate samples from both reactor types were centrifuged at 10,000 x g for 10 min at 10 °C. The supernatant was used after filtration for subsequent analysis, including the measurement of organic acids (OA) and the ratio of total OA to inorganic carbonate (OA/alkalinity ratio, $g_{OA}\ g_{CACO3}^{-1}$) by using a Titration Excellence T90 titrator (Mettler-Toledo GmbH, Switzerland). Volatile fatty acids (VFA), including acetic, propionic, n-butyric, iso-butyric, n-valeric, iso-valeric and caproic acid, were determined using a 5890 series II gas chromatograph (Hewlett Packard, USA) equipped with an HS40 automatic headspace sampler (Perkin Elmer, USA) and an Agilent HP-FFAP column (30 m x 0.32 mm x 0.25 µm) according to a method described elsewhere [23]. The total ammonium-nitrogen concentration (TAN in $g\ L^{-1}$) was determined from 500 µL filtered supernatant diluted with deionized water (1:500) with the Neßler method using a benchtop spectrophotometer DR 3900 (Hach-Lange, Germany).

2.6. Kinetic modeling

Based on different model derivations presented by Brulé et al. (2014) an exponential two-pool one-step model (model C) was used to evaluate the methane production kinetics of the batch experiment. This modelling approach differentiates between rapidly and slowly degradable fractions (two-pool) of the available substrate, as shown in **Fig. 1**.

[insert Figure 1 here]

Thus, four model parameters and constants needed to be adjusted to depict the respective measurement results: the total methane potential S_{BMP} (mL CH_4 g_{VS}^{-1}), the ratio of rapidly degradable substrate to total degradable substrate α (-) and the two first-order reaction constants for the degradation of rapidly degradable substrate k_F (d^{-1}), and slowly degradable substrate k_L (d^{-1}). The model implementation as well as the numeric parameter identification (Levenberg-Marquard algorithm) was performed in the software environment Matlab (Mathworks, USA). Furthermore, the method has been extended to include realistic constraints for each parameter. Thus, the kinetics and biogas potential (k and S) can only accept positive values, whereas the ratio between rapidly and slowly degradable substrate components (α) varies between 0 and 1.

2.7. Simulation of CSTR process

To predict the gas production in continuous operation mode by using a CSTR the basic model structure needs to be translated into a general set of differential equations. Considering a constant reaction volume the change of each component in time can be described by the respective mass added and removed during substrate feeding (input and output) as well as the amount involved in anaerobic degradation (first-order kinetics), according to the following equations.

$$\frac{\mathrm{dm_F}}{\mathrm{dt}} = \alpha \cdot \dot{\mathrm{m}}_{\mathrm{DS}} - \mathrm{D} \cdot \mathrm{m_F} - \mathrm{m_F} \cdot \mathrm{k_F} \qquad (1)$$

Where:

- m_F - mass of rapidly degradable substrate components (g);
- α - ratio of rapidly degradable substrate to total degradable substrate;
- $\dot{m}_{DS}$ - mass flow of digestible solids (feed) (g d^{-1});

- *D* - dilution rate (d^{-1});
- m_F - mass of rapidly degradable substrate components (g);
- k_F - first-order reaction constant of rapidly degradable substrate components (d^{-1});

$$\frac{dm_L}{dt} = (1-\alpha) \cdot \dot{m}_{DS} - D \cdot m_L - m_L \cdot k_L \qquad (2)$$

Where:

- m_L - mass of slowly degradable substrate components (g);
- k_L - first-order reaction constant of slowly degradable substrate components (d^{-1});

$$\frac{dV_{CH_4}}{dt} = Y_{CH_4} \cdot (m_F \cdot k_F + m_L \cdot k_L) \qquad (3)$$

Where:

- V_{CH4} - produced biogas volume (mL);
- Y_{CH4} - stoichiometric methane yield (mL CH_4 g_{DS}^{-1});

Thus, the calculation of the resulting volume flow of methane $\dot{V}_{CH_4}$ depends on the specific turn over of rapidly and slowly degradable substrate components and the stoichiometric methane potential Y_{CH_4} as described in **Equation (3)**.

Based on the chemical composition **(Table 2)** and the stoichiometric gas potential of characteristic nutrients the theoretical methane potential Y_{CH_4} of SFC can be determined. To account for anaerobically non-degradable substrate components the amount of carbohydrates (XF and NFE) is subtracted by the substrate-specific lignin content of 116 g per kg TS as measured/stated by Janke et al [25]. Assuming that raw proteins and lipids of SFC are completely degradable under anaerobic conditions a maximum degradability quotient (DQ) of 77.8 % VS can be defined **(Table 3)**.

[insert Tables 2-3]

By multiplying the share of each degradable nutrient with its stoichiometric methane or biogas potential (as stated in VDI 4630 [12], minus 5% to account for microbial growth and maintenance) the corresponding gas potential of individual nutrients of SFC can be calculated. Summation of respective potentials yields a maximum theoretical potential of 340 mL methane and 623 mL biogas per g VS **(Table 3)**. Thus, depending on the bioavailability and applied pre-treatment technologies the measured methane yield during batch and continuous operation should always be lower than the estimated potential. Based on a share of 778 g degradable organic material per kg VS and a respective methane potential of 340 mL per g VS the total stoichiometric methane potential of 437 mL methane per g degradable solids (DS) can be derived for application in the following calculations.

The input mass flow of digestible solids $\dot{m}_{DS}$ can be calculated based on the daily mass of fresh matter added and the content of total and volatile solids (TS in kg kg_{FM}^{-1} and VS in kg kg_{TS}^{-1}) as well as the respective digestibility quotient DQ.

$$\dot{m}_{DS} = \dot{m}_{FM} \cdot TS \cdot VS \cdot DQ \qquad (4)$$

Where:

- $\dot{m}_{FM}$ - mass flow of fresh matter (feed) (g d^{-1});
- TS - total solids (% FM or g g_{FM}^{-1});
- VS - volatile solids (% TS or g g_{TS}^{-1});
- DQ - digestibility quotient (g DS g_{VS}^{-1});

The DQ is approximated based on the total methane potential obtained from the BMP test as shown in **Equation (5).**

$$DQ = \frac{S_{BMP}}{Y_{CH_4}} \qquad (5)$$

Where:

- S_{BMP} - estimated total methane potential, based on the simulation of the experimental biochemical methane potential test (mL CH_4 g_{VS}^{-1});

Furthermore, the dilution rate D is defined as the quotient of the total input volume flow and reaction volume or the inverse of the respective HRT.

$$D = \frac{q_{in}}{V_{liq}} = \frac{1}{HRT} \tag{6}$$

Where:

- q_{in} - total input volume flow, including water and NaOH (L d^{-1});
- V_{liq} - reaction volume (mL);
- HRT - hydraulic retention time (d);

For steady-state process conditions, the change of mass in time equals zero.

$$\frac{dm_F}{dt} = \frac{dm_L}{dt} = 0 \tag{7}$$

Thus, the differential **Equations (1-3)** can be solved analytically for the unknown masses of each component in steady-state depending on known parameters or previously calculated masses.

$$m_L = \frac{(1-\alpha)}{D+k_L} \cdot \dot{m}_{DS} \tag{8}$$

$$m_F = \frac{\alpha}{D+k_F} \cdot \dot{m}_{DS} \tag{9}$$

Finally, the respective methane production rate can be determined by inserting the results of **Equation 8-9** into **Equation 3**.

$$\frac{dV_{CH_4}}{dt} = Y_{CH_4} \cdot \dot{m}_{DS} \cdot \left(\frac{\alpha \cdot k_F}{D+k_F} + \frac{(1-\alpha) \cdot k_L}{D+k_L}\right) \tag{10}$$

To depict specific methane production per g VS added, the resulting methane volume flow $\dot{V}_{CH_4}$ needs to be divided by the input mass flow of volatile solids $\dot{m}_{VS}$ per day. Following previous calculations of the amount of degradable solids in **Equation 5**, the daily mass flow of volatile solids can be derived by multiplying the input mass flow of fresh matter with the respective TS and VS content of the substrate mixture (TS in kg kg_{FM}^{-1} and VS in kg kg_{TS}^{-1}).

$$\dot{m}_{VS} = \dot{m}_{FM} \cdot TS \cdot VS \tag{11}$$

The resulting balancing scheme has been implemented as simple spreadsheet calculation in MS-Excel 2016 (Microsoft, USA). All substrate characteristics and kinetic parameters are shown in **Tables 1-3.** Stoichiometric parameters and calculations are summarized in **Table 4.**

[insert Table 4 here]

2.8. Statistical analysis

An analysis of variance (one-way ANOVA) followed by a Tukey pairwise comparison was performed to verify statistical differences ($p < 0.05$) as a result of the thermo-chemical pre-treatment on organic acids, esters, COD, and cumulative methane yields after 5, 10, 15, 20 and 30 days of BMP tests. Additionally, differences on SMP during the semi-continuous experiment were also verified with the same procedure. Both analyses were run with the software Minitab 17 (Minitab, USA).

3. Results and discussion

3.1. Pre-treatment effects on substrate composition

The effects of biomass pre-treatment on SFC composition are shown in **Table 5**. In general, an increased solubilization of organic matter was observed by autoclaving the substrate with or without NaOH. S_{COD} was found to be higher by 5.9% and 40.7% for autoclaved and autoclaved with NaOH pre-treated in comparison to the untreated sample, respectively. Such results confirmed the effectiveness of alkaline reagents to promote hydrolysis as previously reported for sorghum forage, wheat and sugarcane straw [8,26].

[insert Table 5 here]

The autoclaved with NaOH pre-treatment showed limited effects on VFA formation, since three of the major VFAs analyzed (concentration > 1 g L^{-1}), namely *n*-butyric, *i*-butyric and propionic acid, did not show significant differences ($p > 0.05$) among the treatments. The only exception was acetic acid, which showed an increase ($p < 0.05$) in its concentration by autoclaving with or without NaOH, possibly due to the conversion of hydrolyzed hemicelluloses' acetyl groups [7,27].

Furthermore, the formation of potential inhibitors such as furfural or 5-methyl furfural is often related to different pre-treatment methods, especially thermal and chemicals [28]. In this experiment, neither furfural nor 5-methyl furfural were detected, except for the autoclaving with NaOH which presented traces of furfural in two of the three replicates (0.36 ± 0.31 mg L^{-1}).

It is also important to note that due to the process conditions applied during substrate's autoclaving, i.e. 30 min of exposure at 121 °C and 1 bar overpressure, losses of volatile compounds such as VFAs and alcohols often found in ensiled biomass can occur [29]. In this case, as a result of the autoclave-based pre-treatment, significant losses ($p < 0.05$) of the alcohols 1-butanol, 1-propanol and 2-butanol were detected. Such losses of organic matter, however, are considered as of a minor interest, since their concentration corresponded to less than 1% of the total VFA measured.

3.2. Biochemical methane potential tests

The methane yields obtained from the BMP tests and fitted to the two-pool one-step model are shown in **Table 6** and **Fig. 2**. In general, the utilized model depicted the methane progression of the BMP tests with a high degree of precision ($R^2 > 0.99$). From the beginning to the end of the experiment significant differences in S_{BMP} were observed among the treatments ($p < 0.05$), especially between untreated (232 ± 2.83 mL g_{VS}^{-1}) and autoclaved with NaOH pre-treatment (251 ± 6.85 mL g_{VS}^{-1}). Interestingly, in a previous experiment our group reported a much higher value of 317 mL g_{VS}^{-1} for SFC also pre-treated with 6 g NaOH/100g SFC_{FM} during 30 minutes but under mild temperature of 45 °C and atmospheric pressure [2]. In this case, the lower value obtained by the current experiment could be explained by different reasons: (a) seasonal variations in substrate composition, in particular the non-fiber carbohydrates fraction; (b) eventual losses of organic matter during the process of autoclaving; and/or (c) overestimation in the previous study due to the uncorrected dry matter content based on TS determination by oven drying [5,29,30]. In contrast, another study using the same charge of SFC as in the current experiment presented an equal S_{BMP} to the untreated SFC of 231.3 ± 10.6 mL g_{VS}^{-1}, demonstrating the high reproducibility of the BMP tests [31].

[insert Table 6 and Figure 2 here]

The pre-treatment based only on autoclaving showed a 3.87% lower S_{BMP} than the untreated sample. Such slight, but significant variation ($p < 0.05$), can be explained by possible losses of organic matter during the autoclave-based pre-treatment (e.g. alcohols previously discussed) and/or the higher standard deviation found in the experimental data of the autoclaved pre-treatment (± 11.3 mL g_{VS}^{-1}) in comparison to the untreated (± 1.73 mL g_{VS}^{-1}) **(Fig. 3)**. In case the evaporation of alcohols during autoclaving played a major role, the saponification effect provided by using the autoclaved with NaOH pre-treatment has compensated these losses of organic matter since autoclaving with NaOH resulted in the highest performance among the treatments.

[insert Figure 3 here]

In fact, the autoclaved with NaOH pre-treatment improved not only the S_{BMP} of SFC but also accelerated the AD process since the ratio of rapidly degradable substrate to total degradable substrate (α value for constant k_F of 0.86 d^{-1} and k_L of 0.08 d^{-1} in **Table 6**) increased from 0.45 (untreated and autoclaved) to 0.68 (autoclaved with NaOH). This improved overall performance could suggest different benefits for practical applications in a CSTR under semi-continuous feeding mode: (a) allow the reduction in HRT needed to convert the same amount of organic matter as for the untreated SFC and/or (b) increased conversion of organic matter to methane (i.e. higher methane yield) [8].

3.3. Semi-continuous experiment

3.3.1. Start-up phase

For the start-up phase of the semi-continuous experiment (days 0-15) the initial OLR was set to 2.1 g_{VS} L^{-1} d^{-1} and gradually increased to 3.2 g_{VS} L^{-1} d^{-1}, while the HRT was decreased from 38.5 days to 25 days. The start-up of anaerobic reactors is considered as a critical phase during biogas production since often it is subjected to risks of process unbalance or even failure [13]. During this period of the experiment no accumulation of intermediate metabolites was observed (VFA concentration < 50 mg L^{-1}), resulting in a constant pH value of around 7.29 ± 0.02 in the reactors **(Fig. 4)**. These results indicated a proper adaptation of the microbial community to the new substrate resulting in a successful start-up of the AD process [14].

[insert Figure 4 here]

Furthermore, a trend of higher TAN concentration could be observed at day 14 in the reactor receiving SFC autoclaved with NaOH (1.60 g L^{-1}) in comparison to the other reactors (average of 1.15 g L^{-1}). Such higher TAN value can be explained by the following reasons: (a) the utilized HNO_3 to neutralize the substrate after alkaline pre-treatment is converted to NO_3^- in aqueous solutions followed by nitrate/nitrite ammonification via dissimilatory nitrate reduction to ammonium (DNRA); and (b) the additional solubilized organic matter (protein fraction) caused by SFC pre-treatment resulted in the formation of un-ionized ammonia (NH_3), ionized ammonium (NH_4^+), carbamate (NH_2COO^-) and mineral struvite ($MgNH_4PO_4x6H_2O$), increasing TAN concentration when SFC autoclaved with NaOH is used [32,33].

In fact, the higher performance of the reactor with autoclaved with NaOH pre-treated SFC observed from the BMP tests was confirmed during this phase of the semi-continuous experiment. In this case, due to the pre-treatment an increase by 18% ($p < 0.05$) in SMP was found, while only autoclaving the substrate reduced the SMP by 4.4% ($p > 0.05$) in comparison to the reactor receiving untreated substrate **(Fig. 5)**.

[insert Figure 5 here]

3.3.2. First hydraulic retention time

After the start-up phase the OLR and HRT were kept at 3.2 g_{VS} L^{-1} d^{-1} and 25 d, respectively, until the end of the experiment. During the first 25 days (1st HRT) the trend of higher TAN values in the reactor with SFC autoclaved with NaOH (1.46 ± 0.09 g L^{-1}) than in the other reactors (1.16 ± 0.04 g L^{-1}) proceeded. As a result, the pH was slightly increased to an average value of 7.37 ± 0.05 in the reactor receiving substrate autoclaved with NaOH, while the pH values of the other two reactors were kept at 7.30 ± 0.02. Such difference is explained by the ammonia buffering system caused by the equilibrium of ionized ammonium and un-ionized ammonia in aqueous solutions ($NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$). Thus, for each additional mole of NH_4^+ produced as a side effect of the autoclaved with NaOH pre-treatment, one mole of OH^- is released resulting in a higher pH value in the solution [34].

Interestingly, the OA/alkalinity ratio showed higher values for the reactor where the autoclaved with NaOH pre-treatment was applied, even though no VFA accumulation was found in the reactors. In this case, the use of NaOH for substrate pre-treatment followed by HNO_3 might have altered the balance between hydroxides (OH^-) and hydrogen ions (H^+) resulting in such unexpected OA/alkalinity ratio for the reactor receiving substrate autoclaved with NaOH.

Nevertheless, this reactor presented again a higher SMP ($p < 0.05$) in comparison to the reactors receiving untreated and autoclaved SFC. This time, however, a lower difference of 8.6% was found by comparing the autoclaved with NaOH pre-treatment (237.5 ± 12.1 mL g_{VS}^{-1}) with the reactor receiving untreated SFC (218.6 ± 19.9 mL g_{VS}^{-1}). In the meantime, the reactor with autoclaved SFC presented 3.5% higher SMP (226.3 ±

15.0 mL g_{VS}^{-1}) than the untreated ($p < 0.05$), which is a different behavior than found during the start-up phase as well as from the BMP tests **(Figure 6)**.

[insert Figure 6]

3.3.3. Second hydraulic retention time

During the second HRT period (days 41-65) at constant operational conditions (i.e. OLR and HRT) the same trend of TAN concentration found in the end of the start-up phase (day 14) and during the first HRT (days 16-40) was observed. However, at day 48 an accumulation of 1.9 g_{VFA} L^{-1} (95% of acetic acid) was observed in the reactor receiving substrate autoclaved with NaOH. It is well known that high salts levels can cause bacterial cells to dehydrate due to osmotic pressure [11,35]. In this case, the use of NaOH as an alkaline reagent for SFC pre-treatment might have excessively increased the concentration of Na^+, causing a momentary inhibition of the acetate-utilizing methanogens or the syntrophic oxidizing bacteria.

Furthermore, due to the to the previously discussed ammonia buffer system, only a slight variation in the pH value of this reactor was noticed as a result of the VFA accumulation (OA/alkalinity ratio of 0.34). Thus, preventing a harmful drop in the pH value which could have led to a higher process instability or even failure in case the OA/alkalinity ratio would have surpassed the value of 0.45 [36].

During this period of the semi-continuous experiment no significant differences ($p > 0.05$) in SMP were found among the reactors. Therefore, different assumptions could be drawn: (a) differences between reactors with SFC autoclaved and autoclaved with NaOH have always been small (including during BMP tests) and difficult to be depicted; and (b) the VFA accumulation in reactor with SFC autoclaved with NaOH pre-treatment caused instability in biogas production which has increased the standard deviation of the average value. Thus, the differences in the measured SMP could not be distinguished with 95% of confidence level ($p < 0.05$).

3.3.4. Third hydraulic retention time

To provide a reliable assessment of the different pre-treatment methods, the semi-continuous experiment was carried-out until reaching a technical steady-state defined by a period equivalent to 3 times the HRT applied under constant operational conditions (25 d x 3 = 75 d) [12].

During the third HRT period (days 66-90) no accumulation of VFA was observed, leading to constant pH values in all reactors. Additionally, as also observed in the other experimental phases, the higher TAN concentration found in the reactor with SFC autoclaved with NaOH (1.69 ± 0.10 g L^{-1}) than in the other two reactors (1.29 ± 0.10 g L^{-1}) resulted in a higher average pH value of 7.44 ± 0.04 (autoclaved with NaOH pre-treatment) in comparison to 7.31 ± 0.05 found for autoclaved and untreated.

Under steady-state conditions neither the reactor with autoclaved SFC nor the autoclaved with NaOH significantly influenced the SMP of SFC ($p > 0.05$). In fact, autoclaving SFC increased the SMP only by 2.1%, while autoclaving SFC with NaOH resulted in equal SMP than the untreated. Such results are in contrary to the BMP tests previously presented, and also in contrary to other BMP tests reported elsewhere where similar pre-treatment methods showed positive effects on methane yields of ensiled sorghum forage, wheat and sugarcane straw (Janke, 2017, Sambusiti 2013b).

In this case, different results obtained from BMP tests and semi-continuous experiments could be explained by different feeding modes and/or different source, activity and inoculum adaptation to the substrate (Janke, 2017). However, Sambusiti et al. 2013 reported similar benefits of sodium hydroxide pre-pretreatment on ensiled sorghum forage also during semi-continuous experiment under steady-state conditions.

3.4. Batch versus semi-continuous experiments

In general, the results from the BMP tests showed a larger variability from 223 to 251 mL CH_4 g_{VS}^{-1} in comparison to the measurements of the semi-continuous experiment from 218 to 223 mL CH_4 g_{VS}^{-1} (average values during steady-state conditions). Theoretically, the methane potential (S_{BMP}) of a substrate determined during BMP tests should always be higher (or equal) in comparison to the respective methane yield during semi-continuous operation [37].

By neglecting the modelled degradation kinetics, the cumulative methane yields at the last day of the BMP tests are slightly higher or equal ($S_{BMP} \geq 223$ mL CH_4 g_{VS}^{-1}) compared to the methane yield measured during continuous operation ($\dot{V}_{CH_4} \leq 223$ mL CH_4 g_{VS}^{-1}).

For a more comprehensive comparison between batch and semi-continuous operation, a simplified kinetic model based on two first-order reaction kinetics of rapidly and slowly degradable substrate components has been applied. According to the derived balancing equations described in section 2.7 the individual kinetic parameters estimated during batch operation **(Table 4 and 6)** were used for prediction of respective methane yields under semi-continuous feeding in a CSTR.

The estimated methane yield as well as characteristic parameters and individual mass flows of the proposed balancing algorithm according to equation 1 to 11 were summarised in **Table 4**. Due to the kinetic degradation conditions and the effective HRT the resulting methane yield simulated for semi-continuous feeding mode (based on BMP test modelling kinetics) deviates markedly from the semi-continuous experimental results.

Thus, calculations based on model parameters estimated during batch feeding result in a lower methane production of 185 and 174 mL CH_4 g_{VS}^{-1} in comparison to semi-continuous experimental results of 218 and 223 mL CH_4 g_{VS}^{-1} for untreated and autoclave pre-treatment, respectively. This corresponds to absolute or relative difference of 34 and 48 mL CH_4 g_{VS}^{-1} or 15.4 % and 21.7 %.

Only the predicted methane yield for the thermo-chemical pre-treatment coincides with the respective BMP of batch tests (4 mL CH_4 g_{VS}^{-1} absolute and 2 % relative difference) and thereby defines a reasonable connection between methane potential (batch) and yield (semi-continuous operation).

Lower BMP estimates in anaerobic batch tests in comparison to measurements of semi-continuously operated experiments have been reported before. Moeller et al. (2018) measured significantly higher methane yields during semi-continuous AD of triticale. Browne et al. (2014) found as well a greater methane yield during semi-continuous AD of food waste compared to batch tests.

However, only a few studies focus on a direct and model-based comparison of the respective BMP and degradation kinetics estimated in batch and semi-continuous feeding. Batstone et al. (2009) and Jensen et al. (2011) showed that the respective methane potential (substrate degradability) and first-order kinetics from batch tests were not transferable to describe semi-continuous AD of waste sewage sludge.

Furthermore, in previous investigations [10] the authors illustrated that the underlying methane potential during optimized continuous process operation (with N-P-S supplementation) showed higher values in comparison the respective BMP measurements. Kinetics parameters estimated during batch operation did not comply with respective degradation kinetics of semi-continuous operation. Thus, the general validity and transferability of batch test results to evaluate substrate pre-treatments or kinetics degradation behaviour in semi-continuous operation needs to be questioned and investigated in more detail [37].

4. Conclusions

Different total methane potentials (S_{BMP}) were observed in BMP tests as a result of SFC pre-treatment. By simulating the semi-continuous process in a CSTR based on BMP modelled results, the methane yields varied considerably (174-222 mL CH_4 g_{VS}^{-1}). The comparison between simulated and experimental semi-continuous feeding showed a reasonable similarity in methane yields only for the autoclaved with NaOH pre-treatment. In contrast, much higher values were observed for untreated and autoclaved pre-treatment. Such improved yields from untreated and autoclaved pre-treatment prevented the observation of a better performance expected from autoclaved with NaOH during the semi-continuous feeding experiment. Such behaviour is likely explained by a better adaptation of the microbial community to the substrate during long-term semi-continuous feeding experiment, which makes the biomass pre-treatment ineffective under this feeding regime in a single-stage CSTR.

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Abstract

Anaerobic digestion (AD) of sugarcane filter cake (SFC) was investigated by comparing the performance of pre-treatment methods in biochemical methane potential (BMP) tests and semi-continuous experiments. For that, SFC was pre-treated by autoclaving the substrate alone or with sodium hydroxide (NaOH). Experimental data from BMP tests were fitted to a kinetics model and further used for simulating the AD process in a continuous stirred-tank reactor (CSTR). BMP tests showed differences ($p < 0.05$) in total methane potential (S_{BMP}), which have affected methane yields during simulation in a CSTR. Untreated produced 185 mL CH_4 g_{VS}^{-1}, autoclaved pre-treatment 174 mL CH_4 g_{VS}^{-1} and autoclaved with NaOH pre-treatment 222 mL CH_4 g_{VS}^{-1}. Interestingly, such higher performance of autoclaved with NaOH pre-treatment was only observed at earlier stages during semi-continuous feeding experiment. At steady-state no significant differences ($p > 0.05$) in terms of methane yield were observed among the reactors (average of 224 mL CH_4 g_{VS}^{-1}). These results demonstrate that the benefits of pre-treatment could only be observed in BMP tests, which is likely explained by a better adaptation of the microbial community to the substrate during long term semi-continuous experiment, making SFC pre-treatment ineffective in a single-stage CSTR and under this feeding regime.

Keywords: Biomass conversion; degradation kinetics; feeding regime; methane potential.

1. Introduction

The anaerobic digestion (AD) process has been proven to be an alternative biomass conversion pathway to diversify the product portfolio of sugarcane biorefineries by recovering methane-rich biogas, promoting sustainable waste management practices and reducing greenhouse gas (GHG) emissions [1].

Sugarcane filter cake (SFC) is a solid waste generated during the clarification (physical–chemical process) of sugarcane juice before been used for sugar and first generation bioethanol production. SFC is mainly composed of water, inorganic soil particles, residual sugars and small pieces of sugarcane bagasse, which are often intentionally added to improve the permeability during the recovery of sucrose at the rotary vacuum-drum filter [2]. In contrast to the other solid sugarcane waste, namely straw and bagasse, SFC is not a suitable material to be used as fuel in conventional co-generation systems due to its high moisture and mineral contents [3]. The AD of SFC would allow the recovery of a valuable fuel (methane) to be used in different applications, and at the same time maintaining the mineral content (especially phosphorus) of the digestate allowing its proper use as fertilizer on the sugarcane fields [4,5].

Different studies reported the recalcitrance of SFC in AD processes due to its lignocellulosic structure which prevents the action of microorganisms and enzymes [6,7]. Such drawback can potentially result in lower methane yields and longer hydraulic retention times (HRT) directly affecting the profitability of biogas plants in large-scale applications [8].

However, most part of these studies have been limited to assess the effects of biomass pre-treatment on biochemical methane potential (BMP) tests under batch conditions [6–9]. A previous study from our group on the AD of sugarcane straw co-digested with SFC showed that BMP tests did not provide a good estimation of the semi-continuous feeding process in a continuous stirred-tank reactor (CSTR), possibly due to the different source, activity and inoculum adaptation as well as feeding mode [10]. Therefore, only by applying a feeding regime used in large-scale applications (semi-continuous) it is possible to have a proper understanding of the benefits of biomass pre-treatment in terms of enhanced methane yields, faster conversion kinetics and improved mixing in CSTRs.

In addition, in case chemical reagents, such as sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), aqueous ammonia (NH_4OH), are used to catalyze the pre-treatment, inhibitors to the AD process (e.g. Na^+, H_2S and NH_3) could be released resulting in process failure [11]. Thus, reinforcing the necessity of assessing the effects of biomass pre-treatment during a feeding regime used in large-scale, since in BMP tests a higher share of inoculum in relation to the substrate is used, which dilutes inhibitors and mislead proper conclusions [12].

In this study, the effects of SFC autoclaving and autoclaving with NaOH were investigated in batch and semi-continuous feeding mode to assess whether these pre-treatment techniques could provide the same benefits in terms of process stability, degradation kinetics and methane yield. Such approach can provide better understanding on how these pre-treatment techniques occur in practice, resulting in important inputs for process designing leading to a more efficient resource utilization.

2. Material and methods

2.1. Substrate and inoculum

SFC was obtained from a distillery plant in the state of Goiás (Brazil) during the 2014/2015 season, transported to Germany in sealed plastic containers and stored at 4

°C until its use. A German large-scale biogas plant that uses maize silage and cattle manure as substrate provided fresh digestate, which was used as inoculum for the BMP tests and the semi-continuous experiment [13,14].

2.2. Substrate pre-treatment

SFC pre-treatment was carried out in 500 mL glass flasks with an alkaline reagent concentration of 6 g NaOH/100 g SFC based on fresh matter (FM). The substrate total solid (TS) content was 83 g L^{-1}. SFC and NaOH solution was manually mixed and autoclaved for 30 min at 121 °C and 1 bar overpressure in a semi-automatic benchtop autoclave 2540 ML (Tuttnauer, Netherlands). After pre-treatment, SFC was neutralized with nitric acid (HNO_3) and stored at 4 °C until its use.

2.3. Biochemical methane potential tests

The BMP of untreated, autoclaved and autoclaved with NaOH pre-treated SFC was determined according to VDI (2016) using an Automatic Methane Potential Test System II (Bioprocess Control, Sweden) at mesophilic temperature (38 ± 1 °C) for 30 days. Prior to the BMP tests, the inoculum was degassed for 7 days at 38 °C to reduce non-specific biogas production. To prevent inhibition, the ratio of substrate/inoculum ($g_{VS}\ g_{VS}^{-1}$) was set to 0.44 ± 0.09 (i.e. around 2.3 times higher amount of inoculum than substrate based on volatile solids (VS)). The pH value in each batch reactor was measured before and after the BMP tests.

2.4. Semi-continuous experiment

Three lab-scale CSTRs, each with 5 L total volume (3 L working volume), were used for this experiment. The reactors were continuously stirred (100 rpm) using a central stirrer with vertical shaped blades to reduce the formation of floating layers. The operation temperature was kept at mesophilic conditions (38 ± 1 °C) by recirculating hot water through the double-walled reactors.

The experiment was carried-out over 90 consecutive days in two phases (start-up and constant operation) until reaching a technical steady-state after completing 3 turnovers based on the HRT [12]. For comparison, all CSTRs were fed with the same feeding frequency (once per day), organic loading rate (OLR), and HRT, only differing in substrate pre-treatment (untreated, autoclaved and autoclaved with NaOH pre-treated). For the start-up phase (days 0-15), the OLR was gradually increased from 2.1 to 3.2 g_{VS} L^{-1} d^{-1} while the HRT was decreased from 38.5 to 25 d. After this period, the OLR and HRT were kept stable until the end of the experiment at 3.2 g_{VS} L^{-1} d^{-1} and 25 d, respectively. To provide sufficient alkalinity and nitrogen as a nutrient 2 g of urea ($(NH_2)_2CO$) per liter of feeding was daily supplemented to all reactors [15]. In addition, to balance the missing macro (C:S ratio) and micronutrients of the substrate, a nutrient solution based on 0.6 g S, 0.9 g Mn, 4.9 mg Co, 20.9 mg Cu, 16 mg Mo, 12 mg Ni, 5 mg W, 285 mg Zn and 2 mg Se per kg of TS was daily supplemented to the reactors according to [16–18]. Detailed information about the different feeding rates, OLR and HRT in each phase of the experiment is listed in **Table 1**.

[insert Table 1 here]

2.5. Analytical methods

TS and VS and of substrate and digestate were determined by drying the samples for 24 h at 105 °C in a drying oven (Binder, Germany) and further reducing the organic content to ashes for 2 h at 550 °C in a high temperature oven (Carbolite, UK). The TS content of SFC was corrected to account for losses of volatile compounds during oven drying according to Weißbach & Strubelt [19]. The solubilized COD (S_{COD}) of centrifuged SFC after pre-treatment was analyzed using a LCK 014 COD kit (Hach-Lange, Germany) according to the manufacturer's protocol. Crude protein, crude lipids, crude fiber and nitrogen-free extractives of SFC were determined according to Weender method [20]. To determine the macro elements composition of SFC (C, H, N, S), about 30 mg of the substrates and 30 mg of WO_3 were weighted and pressed in an aluminum foil for subsequent burning at 1150 °C catalytically with oxygen. The combustion gases were directed through a reduction tube where NOx was reduced to N_2. The remaining gases (CO_2, H_2O, SO_2) passed through three different adsorption columns and were detected with a thermal conductivity detector (C, H, N) and an infrared spectroscopy detector (S) by using a Vario Macro Cube (Elementar, Germany) [21]. To determine the trace elements composition, dried samples were pre-treated with a mixture of $HNO_3/H_2O_2/HF$ and latter neutralized with H_3BO_3, and the resulting clear solution was analyzed by inductively coupled plasma atomic spectrometry – ICP-OES (ThermoFisher iCAP6200) [22].

The daily biogas production in the CSTRs was measured by a drum-type gas meter TG 05 (Ritter, Germany), and corrected to standard temperature and pressure conditions (273.15 K and 101.325 kPa). The composition of the biogas (CH_4, CO_2, O_2 and H_2S) was measured once a week at the headspace of the CSTRs by using a GA2000 Landfill Gas Analyzer (Geotechnical Instruments, UK). The specific methane production (SMP) is presented in milliliters per g of VS or COD (mL g_{VS}^{-1} or mL g_{COD}^{-1}) and the volumetric methane production (VMP) calculated in liters of methane per liter of reactor per day (L L^{-1} d^{-1}).

Five days per week, the pH value of fresh digestate of the CSTRs was measured immediately after sampling (before feeding) with a pH-electrode Sentix 41 (WTW, Germany). Once a week, digestate samples from both reactor types were centrifuged at 10,000 x g for 10 min at 10 °C. The supernatant was used after filtration for subsequent analysis, including the measurement of organic acids (OA) and the ratio of total OA to inorganic carbonate (OA/alkalinity ratio, $g_{OA}\ g_{CACO3}^{-1}$) by using a Titration Excellence T90 titrator (Mettler-Toledo GmbH, Switzerland). Volatile fatty acids (VFA), including acetic, propionic, n-butyric, iso-butyric, n-valeric, iso-valeric and caproic acid, were determined using a 5890 series II gas chromatograph (Hewlett Packard, USA) equipped with an HS40 automatic headspace sampler (Perkin Elmer, USA) and an Agilent HP-FFAP column (30 m x 0.32 mm x 0.25 µm) according to a method described elsewhere [23]. The total ammonium-nitrogen concentration (TAN in g L^{-1}) was determined from 500 µL filtered supernatant diluted with deionized water (1:500) with the Neßler method using a benchtop spectrophotometer DR 3900 (Hach-Lange, Germany).

2.6. Kinetic modeling

Based on different model derivations presented by Brulé et al. (2014) an exponential two-pool one-step model (model C) was used to evaluate the methane production kinetics of the batch experiment. This modelling approach differentiates between rapidly and slowly degradable fractions (two-pool) of the available substrate, as shown in **Fig. 1**.

[insert Figure 1 here]

Thus, four model parameters and constants needed to be adjusted to depict the respective measurement results: the total methane potential S_{BMP} (mL CH_4 g_{VS}^{-1}), the ratio of rapidly degradable substrate to total degradable substrate α (-) and the two first-order reaction constants for the degradation of rapidly degradable substrate k_F (d^{-1}), and slowly degradable substrate k_L (d^{-1}). The model implementation as well as the numeric parameter identification (Levenberg-Marquard algorithm) was performed in the software environment Matlab (Mathworks, USA). Furthermore, the method has been extended to include realistic constraints for each parameter. Thus, the kinetics and biogas potential (k and S) can only accept positive values, whereas the ratio between rapidly and slowly degradable substrate components (α) varies between 0 and 1.

2.7. Simulation of CSTR process

To predict the gas production in continuous operation mode by using a CSTR the basic model structure needs to be translated into a general set of differential equations. Considering a constant reaction volume the change of each component in time can be described by the respective mass added and removed during substrate feeding (input and output) as well as the amount involved in anaerobic degradation (first-order kinetics), according to the following equations.

$$\frac{\mathrm{dm_F}}{\mathrm{dt}} = \alpha \cdot \dot{\mathrm{m}}_{\mathrm{DS}} - \mathrm{D} \cdot \mathrm{m_F} - \mathrm{m_F} \cdot \mathrm{k_F} \qquad (1)$$

Where:

- m_F - mass of rapidly degradable substrate components (g);
- α - ratio of rapidly degradable substrate to total degradable substrate;
- $\dot{m}_{DS}$ - mass flow of digestible solids (feed) (g d^{-1});

- D - dilution rate (d^{-1});
- m_F - mass of rapidly degradable substrate components (g);
- k_F - first-order reaction constant of rapidly degradable substrate components (d^{-1});

$$\frac{dm_L}{dt} = (1-\alpha)\cdot \dot{m}_{DS} - D\cdot m_L - m_L\cdot k_L \qquad (2)$$

Where:

- m_L - mass of slowly degradable substrate components (g);
- k_L - first-order reaction constant of slowly degradable substrate components (d^{-1});

$$\frac{dV_{CH_4}}{dt} = Y_{CH_4}\cdot (m_F\cdot k_F + m_L\cdot k_L) \qquad (3)$$

Where:

- V_{CH4} - produced biogas volume (mL);
- Y_{CH4} - stoichiometric methane yield (mL CH_4 g_{DS}^{-1});

Thus, the calculation of the resulting volume flow of methane $\dot{V}_{CH_4}$ depends on the specific turn over of rapidly and slowly degradable substrate components and the stoichiometric methane potential Y_{CH_4} as described in **Equation (3)**.

Based on the chemical composition **(Table 2)** and the stoichiometric gas potential of characteristic nutrients the theoretical methane potential Y_{CH_4} of SFC can be determined. To account for anaerobically non-degradable substrate components the amount of carbohydrates (XF and NFE) is subtracted by the substrate-specific lignin content of 116 g per kg TS as measured/stated by Janke et al [25]. Assuming that raw proteins and lipids of SFC are completely degradable under anaerobic conditions a maximum degradability quotient (DQ) of 77.8 % VS can be defined **(Table 3)**.

[insert Tables 2-3]

By multiplying the share of each degradable nutrient with its stoichiometric methane or biogas potential (as stated in VDI 4630 [12], minus 5% to account for microbial growth and maintenance) the corresponding gas potential of individual nutrients of SFC can be calculated. Summation of respective potentials yields a maximum theoretical potential of 340 mL methane and 623 mL biogas per g VS **(Table 3)**. Thus, depending on the bioavailability and applied pre-treatment technologies the measured methane yield during batch and continuous operation should always be lower than the estimated potential. Based on a share of 778 g degradable organic material per kg VS and a respective methane potential of 340 mL per g VS the total stoichiometric methane potential of 437 mL methane per g degradable solids (DS) can be derived for application in the following calculations.

The input mass flow of digestible solids $\dot{\mathrm{m}}_{\mathrm{DS}}$ can be calculated based on the daily mass of fresh matter added and the content of total and volatile solids (TS in kg $\mathrm{kg_{FM}}^{-1}$ and VS in kg $\mathrm{kg_{TS}}^{-1}$) as well as the respective digestibility quotient DQ.

$$\dot{\mathrm{m}}_{\mathrm{DS}} = \dot{\mathrm{m}}_{\mathrm{FM}} \cdot \mathrm{TS} \cdot \mathrm{VS} \cdot \mathrm{DQ} \qquad (4)$$

Where:

- $\dot{m}_{FM}$ - mass flow of fresh matter (feed) (g d^{-1});
- TS - total solids (% FM or g $\mathrm{g_{FM}}^{-1}$);
- VS - volatile solids (% TS or g $\mathrm{g_{TS}}^{-1}$);
- DQ - digestibility quotient (g DS $\mathrm{g_{VS}}^{-1}$);

The DQ is approximated based on the total methane potential obtained from the BMP test as shown in **Equation (5).**

$$\mathrm{DQ} = \frac{\mathrm{S_{BMP}}}{\mathrm{Y_{CH_4}}} \qquad (5)$$

Where:

- S_{BMP} - estimated total methane potential, based on the simulation of the experimental biochemical methane potential test (mL CH_4 g_{VS}^{-1});

Furthermore, the dilution rate D is defined as the quotient of the total input volume flow and reaction volume or the inverse of the respective HRT.

$$D = \frac{q_{in}}{V_{liq}} = \frac{1}{HRT} \qquad (6)$$

Where:

- q_{in} - total input volume flow, including water and NaOH (L d^{-1});
- V_{liq} - reaction volume (mL);
- *HRT* - hydraulic retention time (d);

For steady-state process conditions, the change of mass in time equals zero.

$$\frac{dm_F}{dt} = \frac{dm_L}{dt} = 0 \qquad (7)$$

Thus, the differential **Equations (1-3)** can be solved analytically for the unknown masses of each component in steady-state depending on known parameters or previously calculated masses.

$$m_L = \frac{(1-\alpha)}{D+k_L} \cdot \dot{m}_{DS} \qquad (8)$$

$$m_F = \frac{\alpha}{D+k_F} \cdot \dot{m}_{DS} \qquad (9)$$

Finally, the respective methane production rate can be determined by inserting the results of **Equation 8-9** into **Equation 3**.

$$\frac{dV_{CH_4}}{dt} = Y_{CH_4} \cdot \dot{m}_{DS} \cdot \left(\frac{\alpha \cdot k_F}{D+k_F} + \frac{(1-\alpha) \cdot k_L}{D+k_L}\right) \qquad (10)$$

To depict specific methane production per g VS added, the resulting methane volume flow $\dot{V}_{CH_4}$ needs to be divided by the input mass flow of volatile solids $\dot{m}_{VS}$ per day. Following previous calculations of the amount of degradable solids in **Equation 5**, the daily mass flow of volatile solids can be derived by multiplying the input mass flow of fresh matter with the respective TS and VS content of the substrate mixture (TS in kg kg_{FM}^{-1} and VS in kg kg_{TS}^{-1}).

$$\dot{m}_{VS} = \dot{m}_{FM} \cdot TS \cdot VS \tag{11}$$

The resulting balancing scheme has been implemented as simple spreadsheet calculation in MS-Excel 2016 (Microsoft, USA). All substrate characteristics and kinetic parameters are shown in **Tables 1-3.** Stoichiometric parameters and calculations are summarized in **Table 4.**

[insert Table 4 here]

2.8. Statistical analysis

An analysis of variance (one-way ANOVA) followed by a Tukey pairwise comparison was performed to verify statistical differences ($p < 0.05$) as a result of the thermo-chemical pre-treatment on organic acids, esters, COD, and cumulative methane yields after 5, 10, 15, 20 and 30 days of BMP tests. Additionally, differences on SMP during the semi-continuous experiment were also verified with the same procedure. Both analyses were run with the software Minitab 17 (Minitab, USA).

3. Results and discussion

3.1. Pre-treatment effects on substrate composition

The effects of biomass pre-treatment on SFC composition are shown in **Table 5**. In general, an increased solubilization of organic matter was observed by autoclaving the substrate with or without NaOH. S_{COD} was found to be higher by 5.9% and 40.7% for autoclaved and autoclaved with NaOH pre-treated in comparison to the untreated sample, respectively. Such results confirmed the effectiveness of alkaline reagents to promote hydrolysis as previously reported for sorghum forage, wheat and sugarcane straw [8,26].

[insert Table 5 here]

The autoclaved with NaOH pre-treatment showed limited effects on VFA formation, since three of the major VFAs analyzed (concentration > 1 g L^{-1}), namely *n*-butyric, *i*-butyric and propionic acid, did not show significant differences ($p > 0.05$) among the treatments. The only exception was acetic acid, which showed an increase ($p < 0.05$) in its concentration by autoclaving with or without NaOH, possibly due to the conversion of hydrolyzed hemicelluloses' acetyl groups [7,27].

Furthermore, the formation of potential inhibitors such as furfural or 5-methyl furfural is often related to different pre-treatment methods, especially thermal and chemicals [28]. In this experiment, neither furfural nor 5-methyl furfural were detected, except for the autoclaving with NaOH which presented traces of furfural in two of the three replicates (0.36 ± 0.31 mg L^{-1}).

It is also important to note that due to the process conditions applied during substrate's autoclaving, i.e. 30 min of exposure at 121 °C and 1 bar overpressure, losses of volatile compounds such as VFAs and alcohols often found in ensiled biomass can occur [29]. In this case, as a result of the autoclave-based pre-treatment, significant losses ($p < 0.05$) of the alcohols 1-butanol, 1-propanol and 2-butanol were detected. Such losses of organic matter, however, are considered as of a minor interest, since their concentration corresponded to less than 1% of the total VFA measured.

3.2. Biochemical methane potential tests

The methane yields obtained from the BMP tests and fitted to the two-pool one-step model are shown in **Table 6** and **Fig. 2**. In general, the utilized model depicted the methane progression of the BMP tests with a high degree of precision ($R^2 > 0.99$). From the beginning to the end of the experiment significant differences in S_{BMP} were observed among the treatments ($p < 0.05$), especially between untreated (232 ± 2.83 mL g_{VS}^{-1}) and autoclaved with NaOH pre-treatment (251 ± 6.85 mL g_{VS}^{-1}). Interestingly, in a previous experiment our group reported a much higher value of 317 mL g_{VS}^{-1} for SFC also pre-treated with 6 g NaOH/100g SFC_{FM} during 30 minutes but under mild temperature of 45 °C and atmospheric pressure [2]. In this case, the lower value obtained by the current experiment could be explained by different reasons: (a) seasonal variations in substrate composition, in particular the non-fiber carbohydrates fraction; (b) eventual losses of organic matter during the process of autoclaving; and/or (c) overestimation in the previous study due to the uncorrected dry matter content based on TS determination by oven drying [5,29,30]. In contrast, another study using the same charge of SFC as in the current experiment presented an equal S_{BMP} to the untreated SFC of 231.3 ± 10.6 mL g_{VS}^{-1}, demonstrating the high reproducibility of the BMP tests [31].

[insert Table 6 and Figure 2 here]

The pre-treatment based only on autoclaving showed a 3.87% lower S_{BMP} than the untreated sample. Such slight, but significant variation ($p < 0.05$), can be explained by possible losses of organic matter during the autoclave-based pre-treatment (e.g. alcohols previously discussed) and/or the higher standard deviation found in the experimental data of the autoclaved pre-treatment (± 11.3 mL g_{VS}^{-1}) in comparison to the untreated (± 1.73 mL g_{VS}^{-1}) **(Fig. 3)**. In case the evaporation of alcohols during autoclaving played a major role, the saponification effect provided by using the autoclaved with NaOH pre-treatment has compensated these losses of organic matter since autoclaving with NaOH resulted in the highest performance among the treatments.

[insert Figure 3 here]

In fact, the autoclaved with NaOH pre-treatment improved not only the S_{BMP} of SFC but also accelerated the AD process since the ratio of rapidly degradable substrate to total degradable substrate (α value for constant k_F of 0.86 d^{-1} and k_L of 0.08 d^{-1} in **Table 6**) increased from 0.45 (untreated and autoclaved) to 0.68 (autoclaved with NaOH). This improved overall performance could suggest different benefits for practical applications in a CSTR under semi-continuous feeding mode: (a) allow the reduction in HRT needed to convert the same amount of organic matter as for the untreated SFC and/or (b) increased conversion of organic matter to methane (i.e. higher methane yield) [8].

3.3. Semi-continuous experiment

3.3.1. Start-up phase

For the start-up phase of the semi-continuous experiment (days 0-15) the initial OLR was set to 2.1 g_{VS} L^{-1} d^{-1} and gradually increased to 3.2 g_{VS} L^{-1} d^{-1}, while the HRT was decreased from 38.5 days to 25 days. The start-up of anaerobic reactors is considered as a critical phase during biogas production since often it is subjected to risks of process unbalance or even failure [13]. During this period of the experiment no accumulation of intermediate metabolites was observed (VFA concentration < 50 mg L^{-1}), resulting in a constant pH value of around 7.29 ± 0.02 in the reactors **(Fig. 4)**. These results indicated a proper adaptation of the microbial community to the new substrate resulting in a successful start-up of the AD process [14].

[insert Figure 4 here]

Furthermore, a trend of higher TAN concentration could be observed at day 14 in the reactor receiving SFC autoclaved with NaOH (1.60 g L^{-1}) in comparison to the other reactors (average of 1.15 g L^{-1}). Such higher TAN value can be explained by the following reasons: (a) the utilized HNO_3 to neutralize the substrate after alkaline pre-treatment is converted to NO_3^- in aqueous solutions followed by nitrate/nitrite ammonification via dissimilatory nitrate reduction to ammonium (DNRA); and (b) the additional solubilized organic matter (protein fraction) caused by SFC pre-treatment resulted in the formation of un-ionized ammonia (NH_3), ionized ammonium (NH_4^+), carbamate (NH_2COO^-) and mineral struvite ($MgNH_4PO_4x6H_2O$), increasing TAN concentration when SFC autoclaved with NaOH is used [32,33].

In fact, the higher performance of the reactor with autoclaved with NaOH pre-treated SFC observed from the BMP tests was confirmed during this phase of the semi-continuous experiment. In this case, due to the pre-treatment an increase by 18% ($p < 0.05$) in SMP was found, while only autoclaving the substrate reduced the SMP by 4.4% ($p > 0.05$) in comparison to the reactor receiving untreated substrate **(Fig. 5)**.

[insert Figure 5 here]

3.3.2. First hydraulic retention time

After the start-up phase the OLR and HRT were kept at 3.2 g_{VS} L^{-1} d^{-1} and 25 d, respectively, until the end of the experiment. During the first 25 days (1st HRT) the trend of higher TAN values in the reactor with SFC autoclaved with NaOH (1.46 ± 0.09 g L^{-1}) than in the other reactors (1.16 ± 0.04 g L^{-1}) proceeded. As a result, the pH was slightly increased to an average value of 7.37 ± 0.05 in the reactor receiving substrate autoclaved with NaOH, while the pH values of the other two reactors were kept at 7.30 ± 0.02. Such difference is explained by the ammonia buffering system caused by the equilibrium of ionized ammonium and un-ionized ammonia in aqueous solutions ($NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$). Thus, for each additional mole of NH_4^+ produced as a side effect of the autoclaved with NaOH pre-treatment, one mole of OH^- is released resulting in a higher pH value in the solution [34].

Interestingly, the OA/alkalinity ratio showed higher values for the reactor where the autoclaved with NaOH pre-treatment was applied, even though no VFA accumulation was found in the reactors. In this case, the use of NaOH for substrate pre-treatment followed by HNO_3 might have altered the balance between hydroxides (OH^-) and hydrogen ions (H^+) resulting in such unexpected OA/alkalinity ratio for the reactor receiving substrate autoclaved with NaOH.

Nevertheless, this reactor presented again a higher SMP ($p < 0.05$) in comparison to the reactors receiving untreated and autoclaved SFC. This time, however, a lower difference of 8.6% was found by comparing the autoclaved with NaOH pre-treatment (237.5 ± 12.1 mL g_{VS}^{-1}) with the reactor receiving untreated SFC (218.6 ± 19.9 mL g_{VS}^{-1}). In the meantime, the reactor with autoclaved SFC presented 3.5% higher SMP (226.3 ±

15.0 mL g_{VS}^{-1}) than the untreated ($p < 0.05$), which is a different behavior than found during the start-up phase as well as from the BMP tests **(Figure 6)**.

[insert Figure 6]

3.3.3. Second hydraulic retention time

During the second HRT period (days 41-65) at constant operational conditions (i.e. OLR and HRT) the same trend of TAN concentration found in the end of the start-up phase (day 14) and during the first HRT (days 16-40) was observed. However, at day 48 an accumulation of 1.9 g_{VFA} L^{-1} (95% of acetic acid) was observed in the reactor receiving substrate autoclaved with NaOH. It is well known that high salts levels can cause bacterial cells to dehydrate due to osmotic pressure [11,35]. In this case, the use of NaOH as an alkaline reagent for SFC pre-treatment might have excessively increased the concentration of Na^+, causing a momentary inhibition of the acetate-utilizing methanogens or the syntrophic oxidizing bacteria.

Furthermore, due to the to the previously discussed ammonia buffer system, only a slight variation in the pH value of this reactor was noticed as a result of the VFA accumulation (OA/alkalinity ratio of 0.34). Thus, preventing a harmful drop in the pH value which could have led to a higher process instability or even failure in case the OA/alkalinity ratio would have surpassed the value of 0.45 [36].

During this period of the semi-continuous experiment no significant differences ($p > 0.05$) in SMP were found among the reactors. Therefore, different assumptions could be drawn: (a) differences between reactors with SFC autoclaved and autoclaved with NaOH have always been small (including during BMP tests) and difficult to be depicted; and (b) the VFA accumulation in reactor with SFC autoclaved with NaOH pre-treatment caused instability in biogas production which has increased the standard deviation of the average value. Thus, the differences in the measured SMP could not be distinguished with 95% of confidence level ($p < 0.05$).

3.3.4. Third hydraulic retention time

To provide a reliable assessment of the different pre-treatment methods, the semi-continuous experiment was carried-out until reaching a technical steady-state defined by a period equivalent to 3 times the HRT applied under constant operational conditions (25 d x 3 = 75 d) [12].

During the third HRT period (days 66-90) no accumulation of VFA was observed, leading to constant pH values in all reactors. Additionally, as also observed in the other experimental phases, the higher TAN concentration found in the reactor with SFC autoclaved with NaOH (1.69 ± 0.10 g L^{-1}) than in the other two reactors (1.29 ± 0.10 g L^{-1}) resulted in a higher average pH value of 7.44 ± 0.04 (autoclaved with NaOH pre-treatment) in comparison to 7.31 ± 0.05 found for autoclaved and untreated.

Under steady-state conditions neither the reactor with autoclaved SFC nor the autoclaved with NaOH significantly influenced the SMP of SFC ($p > 0.05$). In fact, autoclaving SFC increased the SMP only by 2.1%, while autoclaving SFC with NaOH resulted in equal SMP than the untreated. Such results are in contrary to the BMP tests previously presented, and also in contrary to other BMP tests reported elsewhere where similar pre-treatment methods showed positive effects on methane yields of ensiled sorghum forage, wheat and sugarcane straw (Janke, 2017, Sambusiti 2013b).

In this case, different results obtained from BMP tests and semi-continuous experiments could be explained by different feeding modes and/or different source, activity and inoculum adaptation to the substrate (Janke, 2017). However, Sambusiti et al. 2013 reported similar benefits of sodium hydroxide pre-pretreatment on ensiled sorghum forage also during semi-continuous experiment under steady-state conditions.

3.4. Batch versus semi-continuous experiments

In general, the results from the BMP tests showed a larger variability from 223 to 251 mL CH_4 g_{VS}^{-1} in comparison to the measurements of the semi-continuous experiment from 218 to 223 mL CH_4 g_{VS}^{-1} (average values during steady-state conditions). Theoretically, the methane potential (S_{BMP}) of a substrate determined during BMP tests should always be higher (or equal) in comparison to the respective methane yield during semi-continuous operation [37].

By neglecting the modelled degradation kinetics, the cumulative methane yields at the last day of the BMP tests are slightly higher or equal ($S_{BMP} \geq 223$ mL CH_4 g_{VS}^{-1}) compared to the methane yield measured during continuous operation ($\dot{V}_{CH_4} \leq 223$ mL CH_4 g_{VS}^{-1}).

For a more comprehensive comparison between batch and semi-continuous operation, a simplified kinetic model based on two first-order reaction kinetics of rapidly and slowly degradable substrate components has been applied. According to the derived balancing equations described in section 2.7 the individual kinetic parameters estimated during batch operation **(Table 4 and 6)** were used for prediction of respective methane yields under semi-continuous feeding in a CSTR.

The estimated methane yield as well as characteristic parameters and individual mass flows of the proposed balancing algorithm according to equation 1 to 11 were summarised in **Table 4**. Due to the kinetic degradation conditions and the effective HRT the resulting methane yield simulated for semi-continuous feeding mode (based on BMP test modelling kinetics) deviates markedly from the semi-continuous experimental results.

Thus, calculations based on model parameters estimated during batch feeding result in a lower methane production of 185 and 174 mL CH_4 g_{VS}^{-1} in comparison to semi-continuous experimental results of 218 and 223 mL CH_4 g_{VS}^{-1} for untreated and autoclave pre-treatment, respectively. This corresponds to absolute or relative difference of 34 and 48 mL CH_4 g_{VS}^{-1} or 15.4 % and 21.7 %.

Only the predicted methane yield for the thermo-chemical pre-treatment coincides with the respective BMP of batch tests (4 mL CH_4 g_{VS}^{-1} absolute and 2 % relative difference) and thereby defines a reasonable connection between methane potential (batch) and yield (semi-continuous operation).

Lower BMP estimates in anaerobic batch tests in comparison to measurements of semi-continuously operated experiments have been reported before. Moeller et al. (2018) measured significantly higher methane yields during semi-continuous AD of triticale. Browne et al. (2014) found as well a greater methane yield during semi-continuous AD of food waste compared to batch tests.

However, only a few studies focus on a direct and model-based comparison of the respective BMP and degradation kinetics estimated in batch and semi-continuous feeding. Batstone et al. (2009) and Jensen et al. (2011) showed that the respective methane potential (substrate degradability) and first-order kinetics from batch tests were not transferable to describe semi-continuous AD of waste sewage sludge.

Furthermore, in previous investigations [10] the authors illustrated that the underlying methane potential during optimized continuous process operation (with N-P-S supplementation) showed higher values in comparison the respective BMP measurements. Kinetics parameters estimated during batch operation did not comply with respective degradation kinetics of semi-continuous operation. Thus, the general validity and transferability of batch test results to evaluate substrate pre-treatments or kinetics degradation behaviour in semi-continuous operation needs to be questioned and investigated in more detail [37].

4. Conclusions

Different total methane potentials (S_{BMP}) were observed in BMP tests as a result of SFC pre-treatment. By simulating the semi-continuous process in a CSTR based on BMP modelled results, the methane yields varied considerably (174-222 mL CH_4 g_{VS}^{-1}). The comparison between simulated and experimental semi-continuous feeding showed a reasonable similarity in methane yields only for the autoclaved with NaOH pre-treatment. In contrast, much higher values were observed for untreated and autoclaved pre-treatment. Such improved yields from untreated and autoclaved pre-treatment prevented the observation of a better performance expected from autoclaved with NaOH during the semi-continuous feeding experiment. Such behaviour is likely explained by a better adaptation of the microbial community to the substrate during long-term semi-continuous feeding experiment, which makes the biomass pre-treatment ineffective under this feeding regime in a single-stage CSTR.

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Tables and figures

Table 1. Overview of the semi-continuous experiment.

Reactor	**Phase**	**Period (d)**	**Substrate (g d^{-1})**	**Water (mL d^{-1})**	**NaOH (mL d^{-1})**	**HNO_3 (mL d^{-1})**	**NS (mL d^{-1})**	**HRT (d)**	**OLR (g_{VS} L^{-1} d^{-1})**
Untreated	Start-up	0-8	35	41.7	-	-	1.3	38.5	2.1
		09-15	45	53.4	-	-	1.6	30.0	2.7
	Constant operation	16-90	54	64	-	-	2.0	25.0	3.2
Autoclaved	Start-up	0-8	35	41.7	-	-	1.3	38.5	2.1
		09-15	45	53.4	-	-	1.6	30.0	2.7
	Constant operation	16-90	54	64	-	-	2.0	25.0	3.2
Autoclaved with NaOH	Start-up	0-8	35	8	30	3.7	1.3	38.5	2.1
		09-15	45	11.4	37.4	4.6	1.6	30.0	2.7
	Constant operation	16-90	54	13.5	45	5.5	2.0	25.0	3.2

NaOH – sodium hydroxide solution (1M).

HNO_3 – nitric acid solution (65%).

NS – nutrients solution based on 0.6 g S, 0.9 g Mn, 4.9 mg Co, 20.9 mg Cu, 16 mg Mo, 12 mg Ni, 5 mg W, 285 mg Zn and 2 mg Se per kg of substrate TS.

HRT – hydraulic retention time.

OLR – organic loading rate.

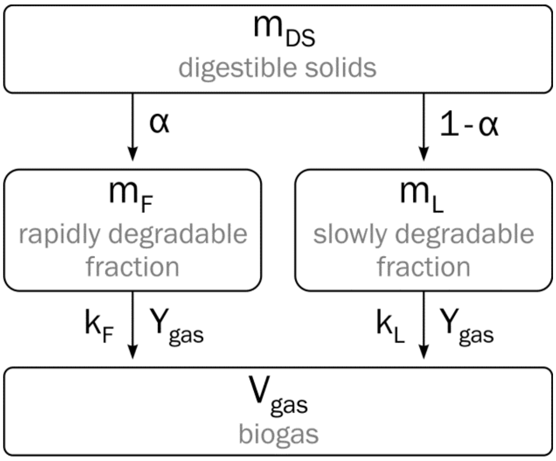

Fig. 1. Components and parameters of the utilized model structure – model C (adopted from Brulé et al. (2014)).

Table 2. Composition of SFC used during the BMP tests and the semi-continuous experiments.

Parameters	**SFC**	**Units**
Total solids (TS)	34.9 ± 0.61	% FM
Volatile solids (VS)	52.0 ± 0.65	% TS
Carbon (C)	48.6 ± 2.01	% TS
Nitrogen (N)	2.44 ± 0.08	% TS
Phosphorus (P)	0.49 ± 0.04	% TS
Sulfur (S)	0.18 ± 0.01	% TS
Iron (Fe)	30,260 ± 1,295.1	mg kg_{TS}^{-1}
Calcium (Ca)	20,304 ± 1,094.1	mg kg_{TS}^{-1}
Sodium (Na)	22.1 ± 2.69	mg kg_{TS}^{-1}
Potassium (K)	1,642 ± 32.45	mg kg_{TS}^{-1}
Magnesium (Mg)	3,436 ± 68.17	mg kg_{TS}^{-1}
Nickel (Ni)	17.9 ± 0.18	mg kg_{TS}^{-1}
Cobalt (Co)	5.08 ± 0.47	mg kg_{TS}^{-1}
Molybdenum (Mo)	*n.d*	mg kg_{TS}^{-1}
Tungsten (W)	*n.d*	mg kg_{TS}^{-1}
Manganese (Mn)	598 ± 49.2	mg kg_{TS}^{-1}
Copper (Cu)	59.1 ± 0.75	mg kg_{TS}^{-1}
Selenium (Se)	0.09 ± 0.02	mg kg_{TS}^{-1}
Zinc (Zn)	115 ± 4.64	mg kg_{TS}^{-1}
Crude protein (XP)	125 ± 1.14	g kg_{TS}^{-1}
Crude lipids (XL)	50.7 ± 1.45	g kg_{TS}^{-1}
Crude fiber (XF)	225 ± 5.92	g kg_{TS}^{-1}
Nitrogen-free extracts (NFE)	120 ± 10.7	g kg_{TS}^{-1}
Ash	477 ± 2.23	g kg_{TS}^{-1}

Note: values are presented in mean (*n*=3); ± represents the standard deviation

FM – fresh matter.

TS – total solids.

n.d – not detected.

Table 3. Degradable components and stoichiometric gas potential of SFC.

	degradable components			stoichiometric gas potential					
	SFC [a, b]			VDI 4630 [c]			SFC		
	total	**degradable**	**DQ**	**methane**	**biogas**	**c_{CH4}**	**methane**	**biogas**	**c_{CH4}**
	(g kg^{-1} VS)	(g kg^{-1} VS)	(%VS)	(mL g^{-1} DVS)	(mL g^{-1} DVS)	(%)	(mL g^{-1} VS)	(mL g^{-1} VS)	(%)
carbohydrates	664	442	66.6	356	713	50.0	158	315	50.0
proteins	239	239	100.0	377	753	50.0	90	180	50.0
lipids	97	97	100.0	951	1321	72.0	92	128	72.0
total	**1000**	**778**	**77.8**	**437**	**801**	**54.5**	**340**	**623**	**54.5**

[a] Composition of characteristic nutrients according to **Table 2** (carbohydrates = XF + NFE).

[b] Assuming lignin is the only non-degradable substrate component. Based on a lignin content of 116 g kg^{-1} TS as measured by Janke et al. (2015).

[c] Stoichiometric biogas potential as stated in VDI 4630 (2016), considering 5% for microbial growth and maintenance.

DQ = degradability quotient

DS = degradable solids

SFC = Sugarcane filter cake

VS = volatile solids

c_{CH4} = concentration of methane

Table 4. Calculation results during mass balancing of individual experimental setups.

Parameters	**Untreated**	**Autoclaved**	**Autoclaved with NaOH**	**Units**
$\dot{m}_{FM}$	120.0	120.0	120.0	g d^{-1}
TS	15.7	15.7	15.7	% FM
VS	52.0	52.0	52.0	% TS
S_{BMP}	232	223	251	mL CH_4 g_{VS}^{-1}
Y_{CH4}	437	437	437	mL CH_4 g_{VS}^{-1}
DQ	0.53	0.51	0.57	-
$\dot{m}_{oTS}$	9.79	9.79	9.79	g d^{-1}
$\dot{m}_{DS}$	5.20	5.01	5.62	g d^{-1}
α	0.45	0.49	0.77	-
k_F	0.86	0.71	0.59	d^{-1}
k_L	0.08	0.07	0.10	d^{-1}
HRT	25	25	25	d
D	0.04	0.04	0.04	d^{-1}
$\dot{m}_L$	23.8	24.2	9.2	g d^{-1}
$\dot{m}_F$	2.6	3.2	6.9	g d^{-1}
$\dot{V}_{CH4,abs}$	1808	1708	2173	mL CH_4 d^{-1}
$\dot{V}_{CH4,spez}$	185	174	222	mL CH_4 g_{VS}^{-1}
$\dot{V}_{CH4,exp}$	218	223	218	mL CH_4 g_{VS}^{-1}
abs. Diff	34	48	-4	mL CH_4 g_{VS}^{-1}
rel. Diff	15.38	21.73	-2.04	mL CH_4 g_{VS}^{-1}

Table 5. Effects of the biomass pre-treatment on SFC composition.

Parameters	**Untreated (mg L^{-1})**	**Autoclaved (mg L^{-1})**	**Autoclaved with NaOH (mg L^{-1})**
1-Butanol	24.8 ± 0.10^{a}	9.78 ± 0.78^{b}	8.26 ± 0.09^{c}
1-Propanol	7.56 ± 0.32^{a}	3.29 ± 0.27^{b}	2.57 ± 0.28^{b}
2-Butanol	80.3 ± 0.31^{a}	25.6 ± 0.28^{b}	19.5 ± 0.28^{c}
2-Propanol	*n.d*	*n.d*	*n.d*
5-Methyl furfural	*n.d*	*n.d*	*n.d*
Acetic acid	4,652 ± 5.36^{b}	4,809 ± 6.21^{a}	4,829 ± 48.0^{a}
Benzoic acid	*n.d*	*n.d*	*n.d*
Decanoic acid	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	0.14 ± 0.01^{a}
Ethanol	*n.d*	*n.d*	*n.d*
Formic acid	7.39 ± 0.22^{c}	38.0 ± 0.36^{b}	286 ± 3.75^{a}
Furfural	*n.d*	*n.d*	0.36 ± 0.31
Hexanoic acid	3.09 ± 0.29^{b}	2.56 ± 0.07^{c}	4.61 ± 0.17^{a}
i-Butyric acid	1,775 ± 2.82^{a}	1,775 ± 3.68^{a}	1,762 ± 13.3^{a}
i-Hexanoic acid	*n.d*	*n.d*	*n.d*
i-Valeric acid	31.3 ± 0.03^{b}	30.3 ± 0.05^{c}	32.6 ± 0.19^{a}
Lactic acid	*n.d*	45.9 ± 10.9^{b}	235 ± 2.0^{a}
Levulinic acid	*n.d*	*n.d*	*n.d*
Nanoic acid	*n.d*	*n.d*	*n.d*
n-Butyric acid	12,050 ± 16.4^{a}	11,885 ± 22.5^{a}	11,985 ± 91.3^{a}
n-Valeric acid	66.7 ± 0.25^{b}	65.2 ± 0.42^{c}	68.9 ± 0.33^{a}
Octanoic acid	0.39 ± 0.03^{b}	0.39 ± 0.01^{b}	1.29 ± 0.03^{a}
Pentyl acetate	*n.d*	*n.d*	*n.d*
Phenylacetic acid	*n.d*	*n.d*	*n.d*
Phenylpropionic acid	10.9 ± 1.44^{a}	9.31 ± 1.03^{a}	9.5 ± 0.10^{a}
Propionic acid	2,990 ± 3.77^{a}	3,007 ± 2.31^{a}	2,982 ± 28.3^{a}
Succinic acid	*n.d*	*n.d*	*n.d*
Total organic acids and esters	21,664 ± 26.4	21,707 ± 34.1	22,226 ± 184
S_{COD}	4,030	4,270	5,670

Note: values are presented in mean (*n*=3), *except for* S_{COD} *(n=1)*; ± represents the standard deviation; $^{a, b, c}$ groups with different letters differ ($p < 0.05$).

n.d – not detected.

Table 6. Results of the biochemical methane potential tests of SFC pre-treated under different conditions and fitted to the two-pool one-step model.

Pre-treatment	**S_{BMP} (mL g_{VS}^{-1})**	**α (-)**	**k_F (d^{-1})**	**k_L (d^{-1})**	**R^2 (-)**	**S_{BMP}^* (mL g_{VS}^{-1})**	**α^* (-)**	**R^{2*} (-)**	**Increase S^* (%)**
Untreated	232 ± 1.73	0.45 ± 0.01	0.86 ± 0.02	0.08 ± 0.01	1.00	232 ± 2.83	0.45 ± 0.01	1.00	-
Autoclaved	223 ± 11.3	0.49 ± 0.02	0.71 ± 0.04	0.07 ± 0.01	1.00	223 ± 10.0	0.45 ± 0.01	1.00	- 3.87
Autoclaved with NaOH	251 ± 6.58	0.77 ± 0.03	0.59 ± 0.11	0.10 ± 0.02	1.00	251 ± 6.85	0.68 ± 0.04	0.99	+ 8.18

Note: values are presented in mean (*n*=3); ± represents the standard deviation.

* optimized values (curve fitting) for constant values of k_F = 0.86 (d^{-1}) and k_L = 0.08 (d^{-1}).

S_{BMP} – total methane potential (approximated at infinite retention time).

α – ratio of rapidly degradable substrate components to total degradable substrate.

k_F – first-order reaction constant for rapidly degradable substrate components.

k_L – first-order reaction constant for slowly degradable substrate components.

R^2 – coefficient of determination.

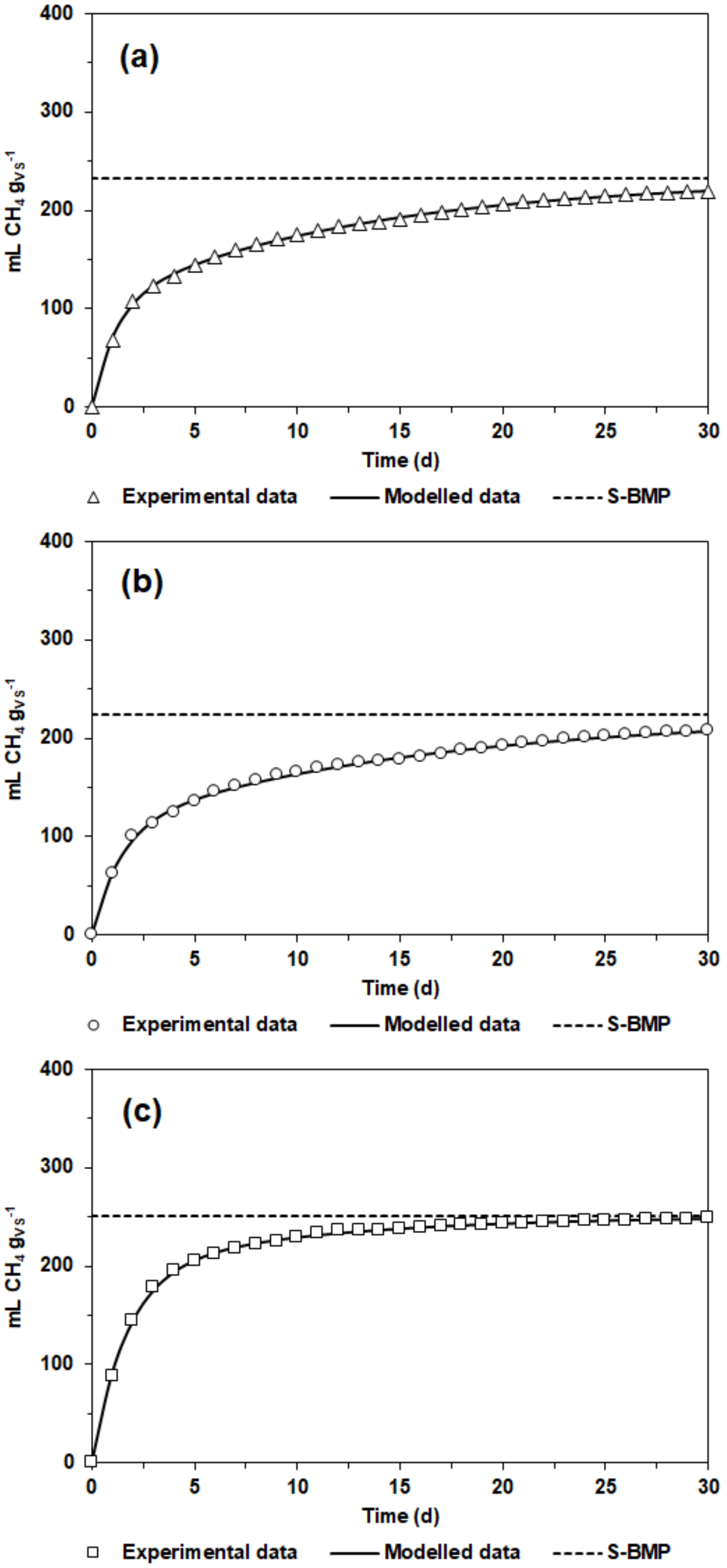

Fig. 2. Cumulative methane yields obtained from the biochemical methane potential tests and fitted to the two-pool one-step model. **(a)** untreated; **(b)** autoclaved and **(c)** autoclaved with NaOH.

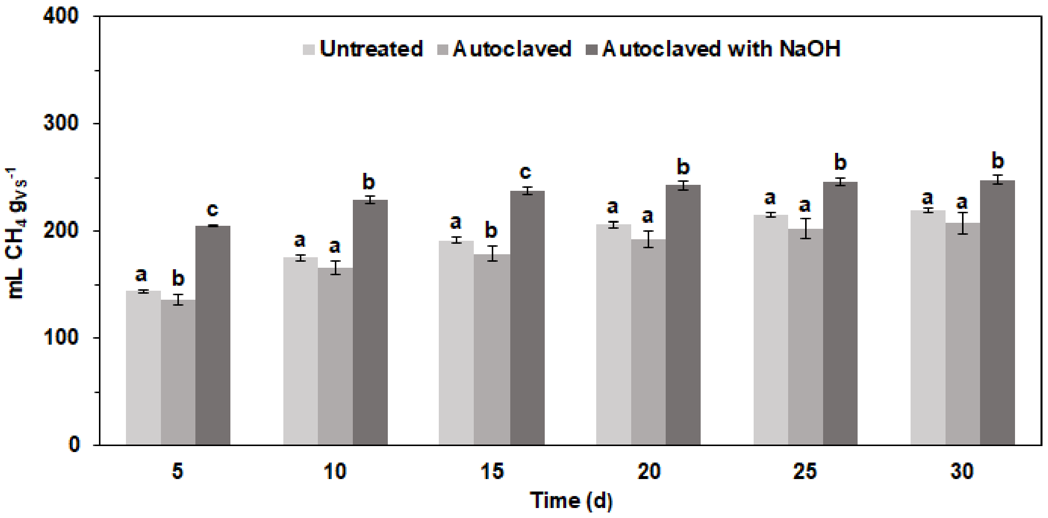

Fig. 3. Tukey's pairwise comparison on the cumulative methane yields after 5, 10, 15, 20, 25 and 30 days of BMP test.

$^{a, b, c}$ groups with different letters differ ($p < 0.05$).

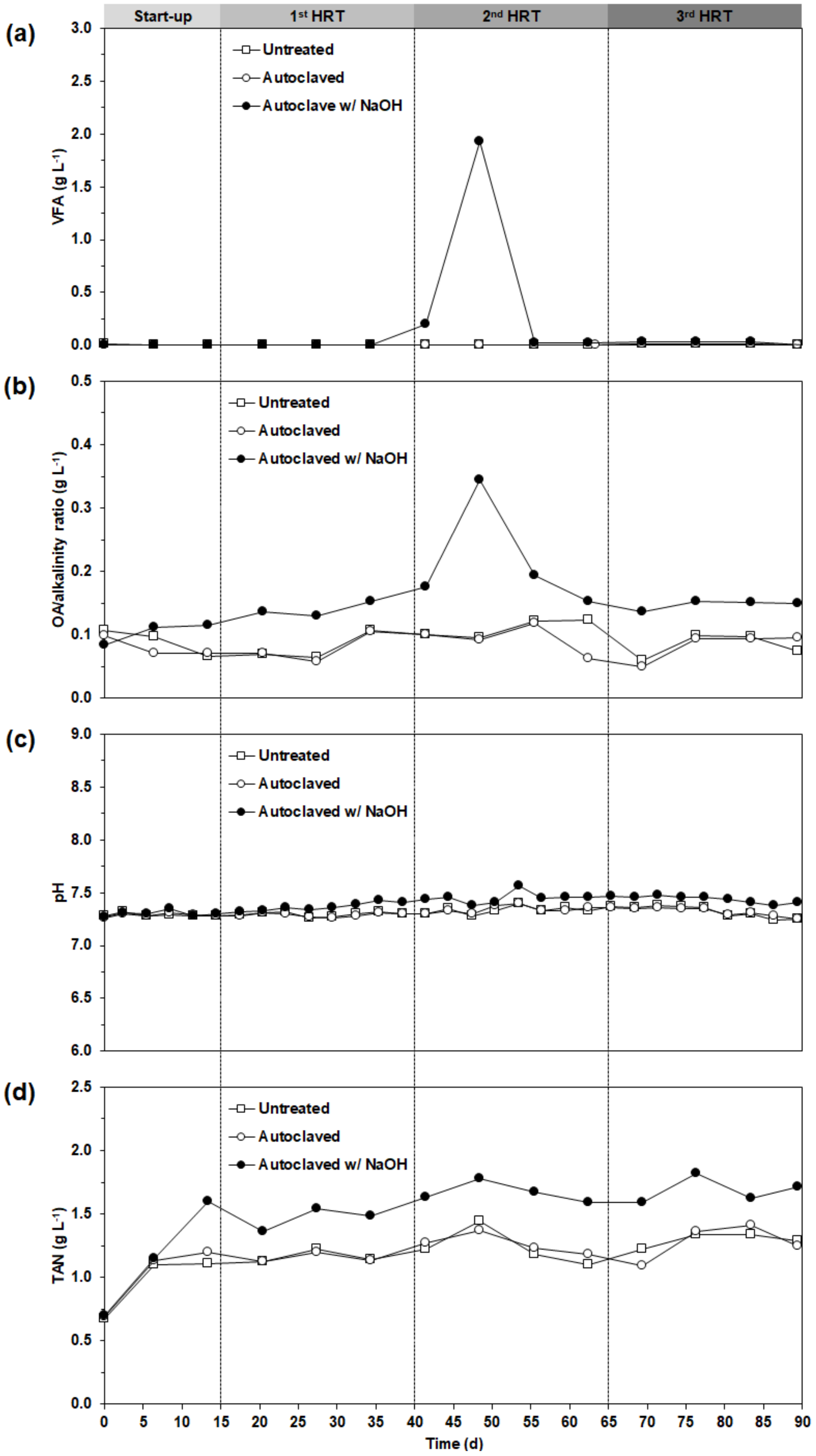

Fig. 4. Process parameters monitored during the semi-continuous experiment. **(a)** volatile fatty acids (VFA); **(b)** ratio of organic acids and total inorganic carbonate (OA/alkalinity ratio); **(c)** pH value and **(d)** total ammonium-nitrogen (TAN).

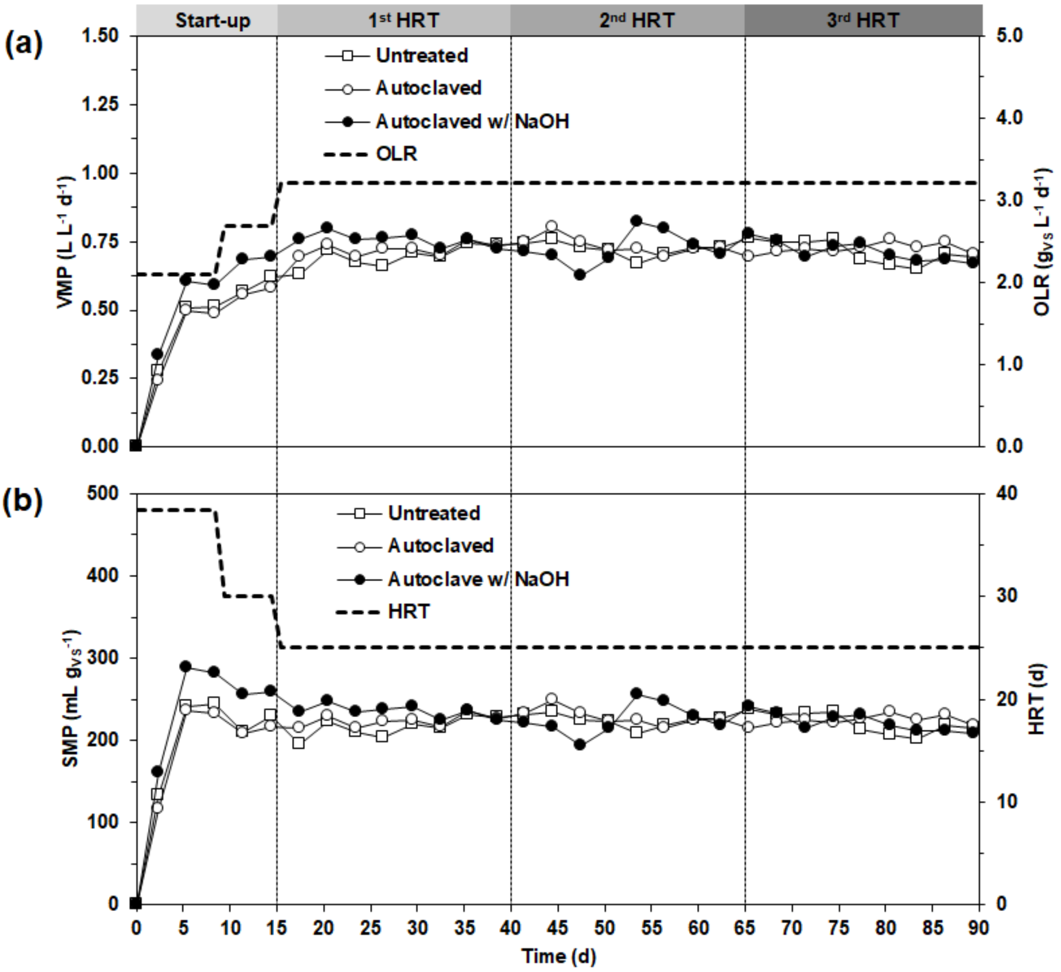

Fig. 5. Monitored methane production during the semi-continuous experiment. **(a)** volumetric methane production (VMP) and organic loading rate (OLR); **(b)** specific methane production (SMP) and hydraulic retention time (HRT).

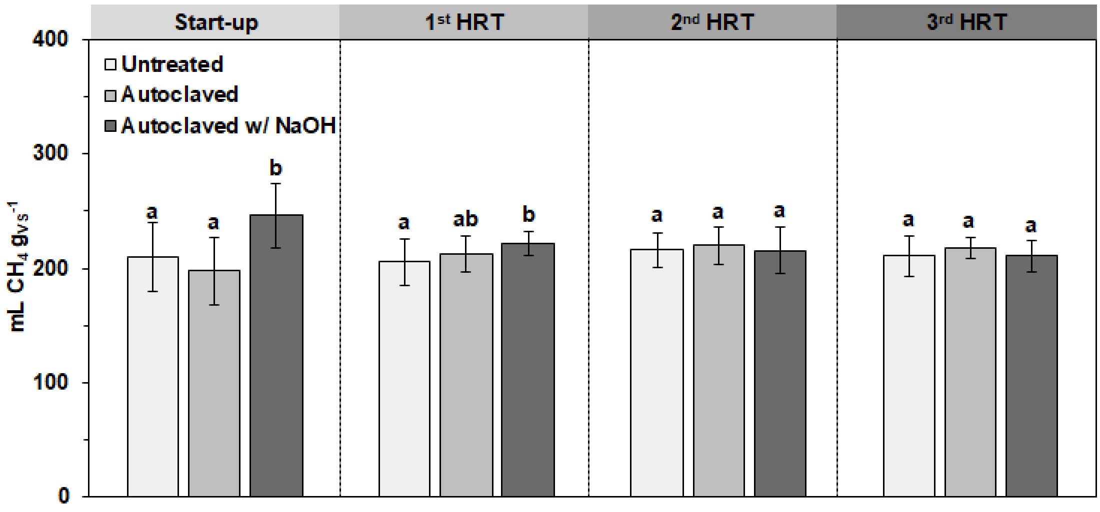

Fig. 6. Tukey's pairwise comparison on the average specific methane production monitored during the semi-continuous experiment.

$^{a,\,b,\,c}$ groups with different letters differ ($p < 0.05$).

Nomenclature

List of abbreviations

AD	anaerobic digestion
BMP	biochemical methane potential
COD	chemical oxygen demand
CSTR	continuous stirred-tank reactor
DNRA	dissimilatory nitrate reduction to ammonium
FM	fresh matter
GHG	greenhouse gas emissions
H^+	Hydrogen ion
HRT	hydraulic retention time
HNO^3	nitric acid
H_2SO_4	sulfuric acid
ICP-OES	inductively coupled plasma atomic spectrometry
$MgNH_4PO_4x6H_2O$	mineral struvite
NaOH	sodium hydroxide
NFC	nitrogen-free extracts
NH_4OH	aqueous ammonia
$(NH_2)_2CO$	urea
NH_3	un-ionized ammonia
NH_2COO^-	carbamate
NH_4^+	ionized ammonium
NO_3^-	nitrate
OLR	organic loading rate
OA	organic acids
OH^-	hydroxide
S_{COD}	solubilized chemical oxygen demand
SFC	sugarcane filter cake
SMP	specific methane production
TAN	total ammonium-nitrogen
TS	total solids
VFA	volatile fatty acids
VMP	volumetric methane production
VS	volatile solids
XF	crude fiber

List of model parameters and symbols

D	dilution rate	d^{-1}
DQ	digestibility quotient	g DS g_{VS}^{-1}
DS	degradable solids	g
HRT	hydraulic retention time	d
k_F	first-order reaction constant of rapidly degradable substrate components	d^{-1}
k_L	first-order reaction constant of slowly degradable substrate components	d^{-1}
m_F	mass of rapidly degradable substrate components	g
m_L	mass of slowly degradable substrate components	g
$\dot{m}_{DS}$	mass flow of digestible solids (feed)	g d^{-1}
$\dot{m}_{FM}$	mass flow of fresh matter (feed)	g d^{-1}
q_{in}	total input volume flow, including water and additives	L d^{-1}
S_{BMP}	total methane potential, based on the simulation of the BMP test	mL CH_4 g_{VS}^{-1}
TS	total solids	% FM or g g_{FM}^{-1}
V_{ch4}	produced methane volume	mL
V_{liq}	reaction volume	mL
VS	volatile solids	% TS or g g_{TS}^{-1}
$\dot{V}_{ch4}$	produced biogas volume flow	mL d^{-1}
Y_{ch4}	stoichiometric methane yield	mL CH_4 g_{DS}^{-1}
α	ratio of rapidly degradable substrate to total degradable substrate	-