Study of the performances of a vinasse mesophilic anaerobic digester behavior submitted to intermittent mixing: Monitoring of the physicochemical properties of the digestate and local samples of the digester

Hélène Caillet, Daniel M Madyira, Laetitia Adelard

Study of the performances of a vinasse mesophilic anaerobic digester behavior submitted to intermittent mixing: monitoring of the physicochemical properties of the digestate and local samples of the digester

Hélène Caillet*[1], Daniel M. Madyira[2], Laetitia Adelard[1]

[1] PIMENT Laboratory, 40 Avenue De Soweto, 97410, Reunion Island, France

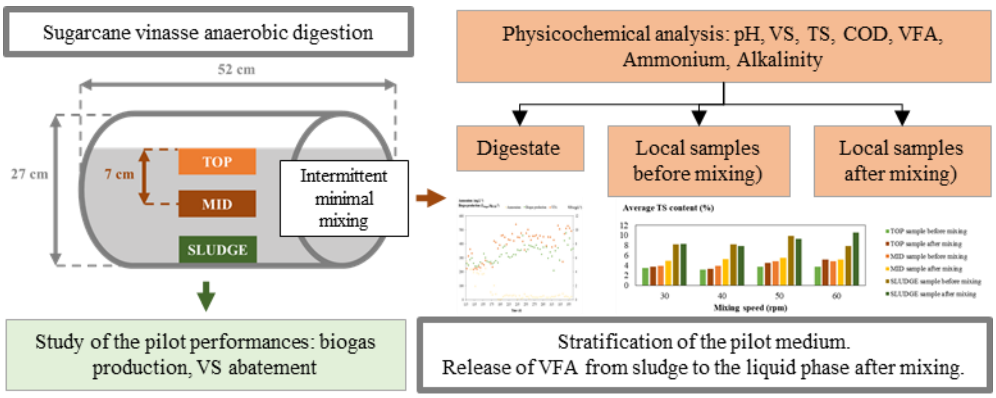
[2] Mechanical Engineering Science Department, Faculty of Engineering and Built Environment, University of Johannesburg, Johannesburg 2006, South Africa

*Corresponding Author: helene.caillet@univ-reunion.fr, Tel: +262 692 08 68 02

Abstract

The aim of this work was to evaluate the response of a vinasse mesophilic anaerobic digester to intermittent mixing. Physicochemical analysis were performed on the digestate, and on three samples collected at three different depths, providing a local insight of the digester before and after mixing. The mixing speed varied from 20 to 60 rpm. The biogas production gradually increased with a maximum production of 477 $L_{CH4}.kg_{COD}^{-1}$ at 60 rpm. Anaerobic digestion was more destabilized at higher mixing speeds, with the increase of biogas production variations. Variations in VFA (Volatile Fatty Acids) and ammonium concentrations were apparent in parallel of biogas production variations. The optimal range of VFA concentration was 8 to 10 $g.L^{-1}$. The VFA / Alk ratio stabilized at the ratio of 0.5 to 0.7. The acidic zones were located in the sludge. A release of VFA from the sludge to the liquid phase was noticed after mixing.

Graphical abstract



Keywords

Anaerobic digestion; sugarcane vinasse; intermittent mixing; physicochemical analysis; biogas production, organic waste treatment.

Highlights

- The optimal ranges of VFA concentrations was 8 to 10 g.L^{-1}.
- The VFA / Alk ratio stabilized between 0.5 and 0.7.
- Stratification of the medium was described through local physicochemical analysis.
- Local investigations showed that the sludge is an acidic zone with high alkalinity.
- Transfers of compounds between sludge and liquid phase were identify.

Statement of novelty

The present work aimed to contribute to the response of a vinasse mesophilic anaerobic digester to intermittent mixing. This one-year experimental study was carried out on the 16 liters anaerobic digester.

The anaerobic digestion of sugarcane vinasse is poorly studied in literature, and not in the case of intermittent mixing, which is a promising strategy, allowing to reduce the power consumption of the industrial units.

This paper provides experimental data on the performances of the digesters in terms of biogas production, and VS abatement. In addition, physicochemical analysis were performed on the digestate, and on three samples collected at three different depths, providing local insight of the digester before and after mixing. This method was used in order to analyze the stratification of the medium.

1. Introduction

Vinasse is produced from distilleries; it is a co-product of the production of alcohols, particularly from sugarcane. Several physicochemical transformations lead to the creation of melanoidins, caramels, flavonoids, tannins and phenolic acids and result in highly polluting products with low pH that are hardly biodegradable (Arimi et al., 2014). Therefore, vinasse is considered as a recalcitrant liquid waste with high organic content (Ramos-Vaquerizo et al., 2018). In Reunion Island and surrounding countries (Mauritius, India, South Africa), the vinasse produced by the many distilleries is a major environmental issue. However, vinasse is also considered as an important raw material for production of biofuels such as biogas or biodiesel.

Reunion Island is a French department localized in the Indian Ocean (55° 32’ E, 21° 08’ S), in a tropical environment. The sugarcane culture is predominant in this region of 2,512 km^2. According to the DAAF (Directorate of Food, Agriculture and Forestry), the surface dedicated to sugarcane corresponds approximately to 52.94 % of the agricultural area (Direction de l’Alimentation, de l’Agriculture et de la Forêt de La Réunion, 2016). In 2015, about 1,896,656 tons of sugarcane were produced, which allowed the production of 201,181 tons of sugar, 80,000 HAP (hectoliters of pure alcohol) and 513,000 tons of bagasse (Direction de l’Alimentation, de l’Agriculture et de la Forêt de La Réunion, 2016). The alcohol is produced from the molasses, resulting from the refining of sugar extracted from sugar beet or sugarcane. In Reunion Island, the molasses are used for the production of rum, 38 % to the Savanna distillery, 59 % to the Rivière du Mât distillery and 3 % to the Isautier distillery (Gosme, 2002). In 2000, approximately 150,000 tons of the vinasses were produced (Gosme, 2002). The rejection of vinasses may cause environmental pollution such as salinity, phytotoxicity, anoxia, eutrophication, death of aquatic life, and many severe health problems due to its high pollutant content (Cruz-Salomón et al., 2017; Ramos-Vaquerizo et al., 2018). Anaerobic digestion (AD) is an appropriate method to reduce the pollutant content of this organic waste while producing biogas (Al-Jabari et al., 2021; Di Maria et al., 2019). This natural process is based on the degradation of organic matter under the action of micro-organisms in the absence of oxygen (Jain and Kalamdhad, 2018). The biogas produced, rich in methane and carbon dioxide, is a source of renewable energy (Khalil et al., 2019; Sun et al., 2015). The biogas has several applications, including fuel production, the substitution of the natural gas or electricity generation (Sahota et al., 2018). Ramos-Vaquerizo et al. (2018) underlined that this treatment is the most environmentally beneficial technology and energy-efficient production.

As vinasse is a recalcitrant waste, many studies have been carried out to evaluate and optimize the AD of sugarcane vinasse. Janke et al. (2015) assessed the kinetics for biogas production from different types of sugarcane waste, in terms of energy potential, degradation rates, and hydraulic retention time (HRT). The vinasses are characterized by an acid pH, and contain important amount of mineral matter (nitrogen, phosphorus, potassium, calcium, magnesium, sodium, *etc*.) and organic matter (organic acids, proteins, polysaccharides, and sugars), melanoidins, hexose degradation products, caramels and polyphenols (Arimi et al., 2014). However, the quantities of each compound vary according to the origin of the vinasses (climatic conditions, sugarcane treatments, fermentation and distillation processes, *etc*.) (Moraes et al., 2015). It is necessary to carry out experimental work on the methanization of different vinasses, since the results obtained on the AD of a given vinasse will not be transposable to another vinasse having a different origin.

At industrial scale, the main parameters are the Organic Loading Rate (OLR), the HRT, and the mixing (intensity, time, and period). Mixing is an important factor because it largely influences the AD process (biogas production, pH, *etc.*) (Kaparaju et al., 2008; Vavilin et al., 2007), as the digestion is based on the contact between the active microbial community and the substrate. The effects of mixing are numerous: the particle flow pattern, the stagnant zones, the liquid velocities, and the shear stress (Singh et al., 2019). The dead zones reduction as well as heat and mass transfer are ensured by homogenization, and sufficient mixing (Mohammadrezaei et al., 2018). However, the biochemical reactions and the microbial community could be destabilized by a too vigorous mixing, resulting in inhibitions: decrease in biogas production or failure of the process (Vavilin and Angelidaki, 2005). Therefore, a proper mixing must be ensured and more research is needed in order to understand the impact of mixing on the process as well as to evaluate the optimized mixing.

Mixing generates energy consumption, directly linked to operating costs. Indeed, up to 50 % of the energy consumption in biogas plant is due to mixing (Wiedemann et al., 2018). The mode of agitation has a major impact on the reduction of electricity consumption. Minimal mixing consists in stirring prior to extraction/feeding phase, and intermittent agitation is any discontinuous agitation. In this context, minimal mixing allows to reduce the energy demand and maintenance cost and to improve the biogas production (Kariyama, 2018). Kaparaju et al. (2008) compared minimal mixing (mixing for 10 min prior to extraction/feeding), intermittent mixing (withholding mixing for 2 h prior to extraction/feeding) and continuous feeding, with three slurry anaerobic digesters. Minimal mixing is a method to homogenize the medium rather than mixing. The highest methane production was obtained with minimal mixing (Kaparaju et al., 2008). This type of mixing is thus promising. Experimental study carried out by Sulaiman et al. (2009) allowed to conclude that the minimal mixing is sufficient to permit good contact between the substrate and the microorganisms, as well as to release the entrapped biogas at the bottom of the digester. In addition, the impact of mixing should be studied on various substrates, and mixing modes, as the proper mixing depends on the nature of the substrate (Lindmark et al., 2014). The results obtained on the slurry cannot be applied to the vinasse, and no work has been done on AD of vinasse with minimal intermittent mixing. Van Hulle *et al.* showed that at pilot scale (120 L) the methane production, due to VFA accumulation, was impacted by the mixing or non-mixing conditions (Van Hulle et al., 2014). Therefore, the analysis of the physicochemical properties of the digestate could contribute to the understanding of the effect of mixing on the process.

The present work aimed to evaluate the response of a vinasse mesophilic anaerobic digester submitted to minimal intermittent mixing over one year. The mixing intensity was gradually increased along the experimental study. The outcomes were the volume of biogas produced, and the physicochemical properties (pH, VFA, ammonium, alkalinity, total solids, and volatile solids) of the digestate and three samples collected at three different depths, providing local experimental data before and after mixing.

2. Material and Methods

2.1. Digestion process and digester

The mesophilic mono-digestion of sugarcane vinasse was studied. The digester has a double jacket in which water is circulated. A water bath with water circulation was used to maintain the water at 37 °C. The anaerobic digester is horizontal and cylindrical with a total volume of 16 L and an operating volume of 13.5 L. The external and internal diameters were respectively 0.27 and 0.21 m and the external tank length is 0.52 m.

The mechanical stirring could be set from 20 to 100 rpm with a step of 10 rpm. The impeller consisted in two rows of two blades spaced apart from 0.165 m. Each blade was 0.09 m length, 0.05 m width, and made of steel sheet of 0.001 m thickness.

The digester was equipped with a security safety valve and a dial for monitoring the temperature of the digestion medium. The bucket counter was used to measure the biogas volume produced with a measuring chamber volume of 3.18 mL, a minimum flow of 1 $mL.h^{-1}$ and a maximum flow of 1 $L.h^{-1}$. The set-up is described on the Fig. 1.A.

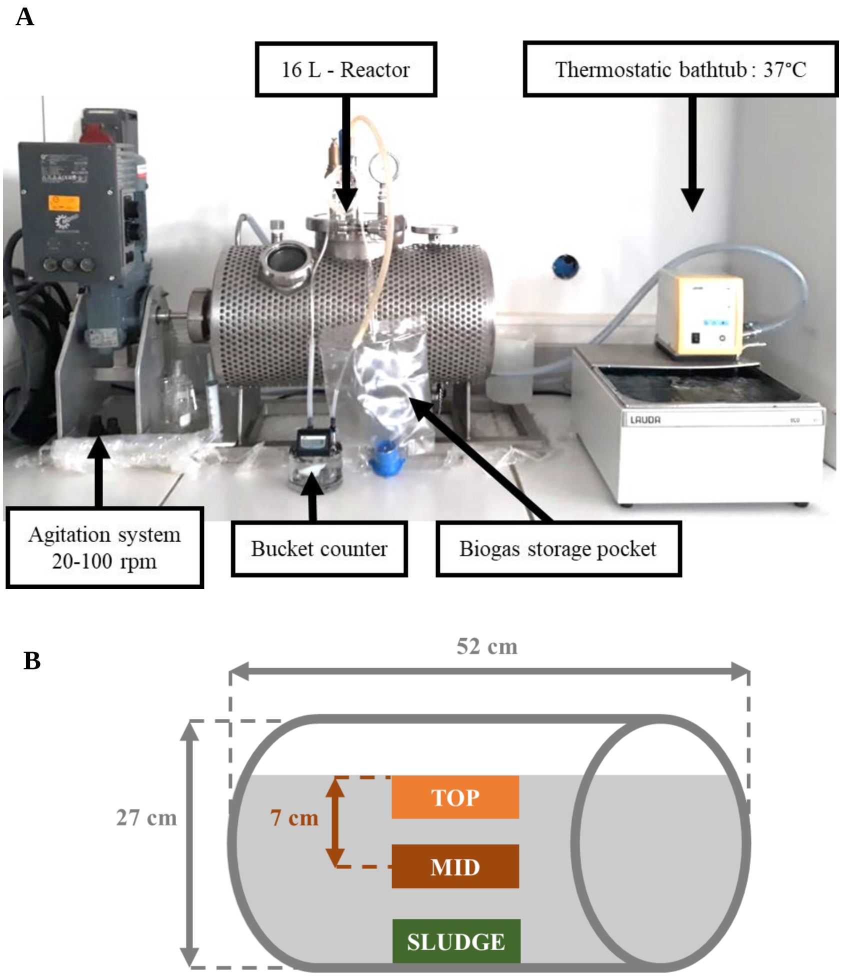


Fig. 1. Anaerobic digester set-up (A) and positions of the local samples (B).

2.2. Substrate, inoculum, and physicochemical analysis

The vinasse studied in this paper came from an industrial distillery. In the distillery, the vinasse was diluted before the AD treatment to optimize the biogas production and avoid the instabilities of the process. In addition, vinasse AD is subject to various inhibitions due to its low carbon content. For this reason, press mud (López González et al., 2017), cellulose and straw (Moraes et al., 2015) were usually used as co-substrates. Furthermore, the low pH of the vinasse could lead to instabilities, and soda solution was added to alkalize the digestion medium (López González et al., 2017). No chemical was added to adjust the physicochemical properties. The vinasse was not diluted with water before feeding, whilst it was done by many authors (Barros et al., 2016; Janke et al., 2016; Moraes et al., 2015).

Prior to this study, the 16-L digester has already been running for 130 days, and the inoculum was already acclimatized to the sugarcane vinasse from the distillery Rivière du Mât (Reunion Island). The experimental data for this period were presented in a previous work (Caillet and Adelard, 2020) and emphasized in the results section. The start-up of the digester and the increase of the organic load were detailed in (Caillet and Adelard, 2020). The same feedstock was used in this work. The substrate and the inoculum were homogenized at 12,000 rpm for 10 min before the characterization tests. The Total Solid (TS) content was obtained after drying 20 g of the samples for 24 h at 105 °C and the Volatile Solid (VS) content after burning the dried samples for 4 h at 550 °C. The VFA, the ammonium, the Chemical Oxygen Demand (COD), the Total Organic Carbon (TOC), the Nitrogen (N), and the alkalinity of the substrate were measured on the input. The chemical tests were conducted using the Hach Lange tests. The biochemical methane potential (BMP) test was carried out in a previous work and the results are provided in supplementary material (Caillet et al., 2019; Caillet and Adelard, 2020).

A 60 mL sample of the digestate was retrieved to obtain a representative sample. In addition, 60 mL local samples were collected before and after mixing at three different depths: at the surface, at 7 cm depth, and at the bottom of the digester. The samples were respectively called TOP, MID, and SLUDGE. The locations are shown on Fig. 1(C). Physicochemical (VFA, ammonium, TS, VS, and alkalinity) analysis were performed on the digestate and the 6 local samples (TOP, MID, and SLUDGE before and after mixing).

The removed VS content was calculated as follows:

$$VS_{\mathrm{Re}moval} = \frac{VS_{digested}}{VS_{added}} * 100 = \frac{VS_{added} - VS_{measured}}{VS_{added}} * 100 \qquad (1)$$

Where VS_{added} is the sum of added VS content in the digester and $VS_{measured}$ is the VS content of the digestate.

2.3. Organic Loading, HRT, and mixing conditions

The digester was fed every four days. At each feeding, 1,500 mL were removed from the digester, and the same volume of vinasse was added. The influent flow rate was 0.375 L.d^{-1}.

The hydraulic retention time (HRT) was 36 days. It was calculated by:

$$HRT = \frac{WV}{Q} \tag{2}$$

Where WV is the working volume (m^3) and Q is the influent flow rate (m^3.d^{-1}).

During the start-up phase, the minimal intermittent mixing condition applied was 15 min at 20 rpm. The mixing coincided with the extraction/feed (every four days just prior to the feeding) (Caillet and Adelard, 2020), as recommended in literature (Kariyama, 2018). Then, the mixing speed was progressively increased from 20 to 60 rpm with a step of 10 rpm. The agitation remained a minimal intermittent mixing in terms of frequency and duration which remain unchanged.

Our experimental study period was approximately one year, so the vinasse was recovered three times along the study at the Distillery Rivière du Mât. As the COD, TS content and therefore the OLR of the vinasse could vary during the sugar campaign, these parameters were evaluated for each time.

2.4. Statistical analysis

The standard deviation (SD) is a measure of the dispersion of a statistical sample. The SD thereby provides additional information to the mean. Indeed, it measures how concentrated the data are around the average. A small SD signifies that the values are concentrated around the average and a large SD reflects many variations. In our work, this value was used to describe the dispersion of the biogas production and the physicochemical properties for each mixing speed.

The SD was calculated as follows:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{n}\left(x_i - \overline{x}\right)^2} \tag{3}$$

Where σ is the SD, N is the number of values, x_i is the quantitative value, and $\overline{x}$ is the average value.

3. Results and Discussion

3.1. Substrate and inoculum characteristics

The physicochemical properties of the inoculum and the vinasse used are given in supplementary material. The average TS and VS contents of the vinasse are respectively 7.85 ± 0.71 % and 5.23 ± 0.48 %. The vinasse is characterized by an acidic pH with a value of 4.74 ± 0.15. The average COD, TOC, VFA, ammonium, and alkalinity are 91.2 ± 5.14 $g_{O2}.L^{-1}$, 30,388 ± 725 $mg.L^{-1}$, 18,646 ± 3 VFA $mg.L^{-1}$, 38.74 ± 5.64 $mg.L^{-1}$, and 1,796 ± 637 $mg_{CaCO3}.L^{-1}$.

The alkalinity (Alk), TOC, COD, ammonium and nitrogen concentrations and the TS and VS content of the vinasse-2 are upper to those of the vinasse-1. Nevertheless, the VFA concentration of the vinasse-2 is lower to those of the vinasse-1. The Alk / COD and C / N ratio of the vinasse samples are close with an average value of 0.02 ± 0.01 and 24.93 ± 2.47. C / N ratio comprised between 20 and 30 was considered in literature as the optimum ratio for AD process because sufficient nitrogen is available for microbial growth and the organic carbon can be degraded (Fricke et al., 2007). The VFA / Alk ratio is more variable with 17.9 for the vinasse-1, 7.59 for the vinasse-2 and 9.27 for the vinasse-3. The vinasse-3 has the highest value of TS and VS content and VFA concentration. In addition, the vinasse-2 has the highest value of COD and ammonium concentration. Globally, the chemical properties of the three vinasse feedstocks are close, but the analysis showed some variations, mostly at the level of VFA concentration and alkalinity with a SD of 3,114 $mg.L^{-1}$ and 637 $mg_{CaCO3}.L^{-1}$ respectively.

The OLR as a function of the sample of vinasse used was studied. The vinasse-1, vinasse-2, and vinasse-3 correspond to the vinasse taken from the Rivière du Mât distillery recovered at intervals of 2 months. The variations of VS content and COD were calculated between the used sample and the previous substrate sample. The variation of OLR in terms of VS content was negligible (3.15 %), whereas the variation in terms of COD was higher (11.50 %) between vinasse-1 to the vinasse-2 samples. Concerning the vinasse-3, the OLR in terms of VS and COD varied respectively by 15.27 and 6.75 %. The average values of OLR in terms of VS and COD were respectively 1.36 ± 0.13 $g_{VS}.L^{-1}.d^{-1}$ and 2.38 ± 0.13 $g_{COD}.L^{-1}.d^{-1}$ with the SD which respectively represented 9.55 and 5.46 % of the average value. In conclusion, the OLR varied non-significantly. The feeding rate and influent concentration of each samples are given in supplementary material.

3.2. Study of the process performance of the digester

The minimum, maximum, median, SD and average biogas production per mixing period are presented in the Fig. 2.A. The boxplots of the TS content and VS content of the digestate for each mixing period are respectively presented in Fig. 2.B and Fig. 2.C. The Fig. 3.A shows the biogas production at different mixing speeds and the variation in biogas production between feeding as a percentage of production variation. Each point corresponds to the production obtained for 1500 mL of vinasse added in the digester. The variations of the VS content and removal VS are shown on the Fig. 3.B. The experimental data are given in supplementary material.

The removal VS was upper than 80 % for the whole study with a maximum value of 97.18 % (Fig. 3.B). Both the removal VS and the VS content gradually increased along the process. The TS solid content increased in function of the time whereas the VS content stabilized from the mixing period of 40 rpm. The dispersion was higher for the VS content than the TS content (Fig. 2.B and Fig. 2.C). The average TS content of the sludge and the vinasse were respectively 3.67 and 7.85 ± 0.71 %. The TS content variation of the digestate varied from 0.31, 0.89, 1.14, 1.11 to 1.60 % with the mixing speeds from 20, 30, 40, 50 to 60 rpm. The VS content of the digestate varied from 0.28, 0.75, 0.85, 0.73 to 1.33 % with the mixing speeds from 20, 30, 40, 50 to 60 rpm. The TS and VS contents increased linearly during the process despite the mixing changes. The TS and VS content respectively increased by 0.01 and 0.005 % per day. This augmentation reflected the accumulation of the solid matters in the digester.

The proportion of methane in biogas for AD of vinasse in mesophilic conditions is 65 % (Arreola-Vargas et al., 2018; Nery, 2018). Considering this percentage, the methane production is presented in supplementary material for different proportions of methane, from 65 to 45 % of methane. The BMP of the vinasse was 185.59 $NL_{CH4}.kg_{COD}^{-1}$. Therefore, the methane production cannot exceed this value. The maximum proportion of methane was 65 % at 20 rpm and 30 rpm, 55 % at 40 rpm, 45 % at 50 rpm, and 50 % at 60 rpm.

The first period (0-114 d) corresponds to the start-up of the reactor. The study of the start-up of the digester was presented in a previous work (Caillet and Adelard, 2020). During this period, the mixing speed was 20 rpm. An important data dispersion on this period was reported with a SD of 106 $L_{biogas}.kg_{COD}^{-1}$ (Fig. 2.A). This important dispersion is logical because this period corresponds to the start-up period of the digester exhibiting significant variations in biogas production. In fact, start-up of anaerobic digester is recognized as a critical phase with important risks of unbalance and failure of the process (Paulose and Kaparaju, 2021). Significant dispersions of biogas production were also observed in the periods 2 (115-161 d) and 6 (272-365 d), corresponding respectively to mixing speeds of 20 and 60 rpm. This results suggest that the biogas production was less stable during these periods. The variations of biogas production in period 2 can be explained by the age of the anaerobic reactor during this period (Bollon, 2012). In fact, the acclimation of microorganisms provides the reactor with resistance to high VFA concentrations (Bollon, 2012). The instabilities in biogas production observed at period 6 could be explained by the higher mixing speed applied during the period 6. The largest dispersions of the TS and VS contents of the digestate were also observed in period 6 (Fig. 2.B and Fig. 2.C). It can be concluded that the digestion process was less stable at the period 6.

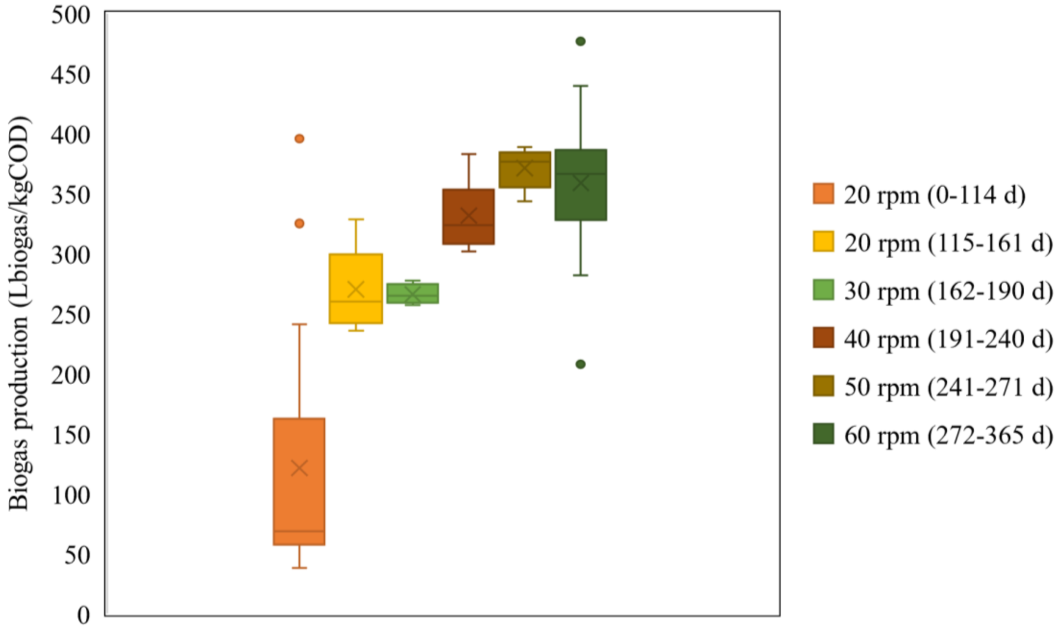
The average biogas production with the mixing speed of 20 rpm and 30 rpm were 268 $L_{biogas}.kg_{COD}^{-1}$ with a SD of 29 and 7 $L_{biogas}.kg_{COD}^{-1}$ respectively. No remarkable variation in biogas production was observed at mixing speed change from 20 to 30 rpm (day 161).

An increase in biogas production was noted at mixing speed change from 30 to 40 rpm (day 190). Indeed, the biogas production ranged from 264 to 383 $L_{biogas}.kg_{COD}^{-1}$ from day 190 to 206, which represented an increase of 45 %. Then, the production slowly decreased, but remained higher than the production obtained for the mixing speed of 30 rpm. This punctual increase in biogas production could be due to the return of undigested material accumulated at the bottom of the digester in the liquid phase, or to the evacuation of the gas bubbles under the action of the agitation. The average biogas production for this period was 332 $L_{biogas}.kg_{COD}^{-1}$. The average production with an agitation of 40 rpm was higher than the production with lower agitation.

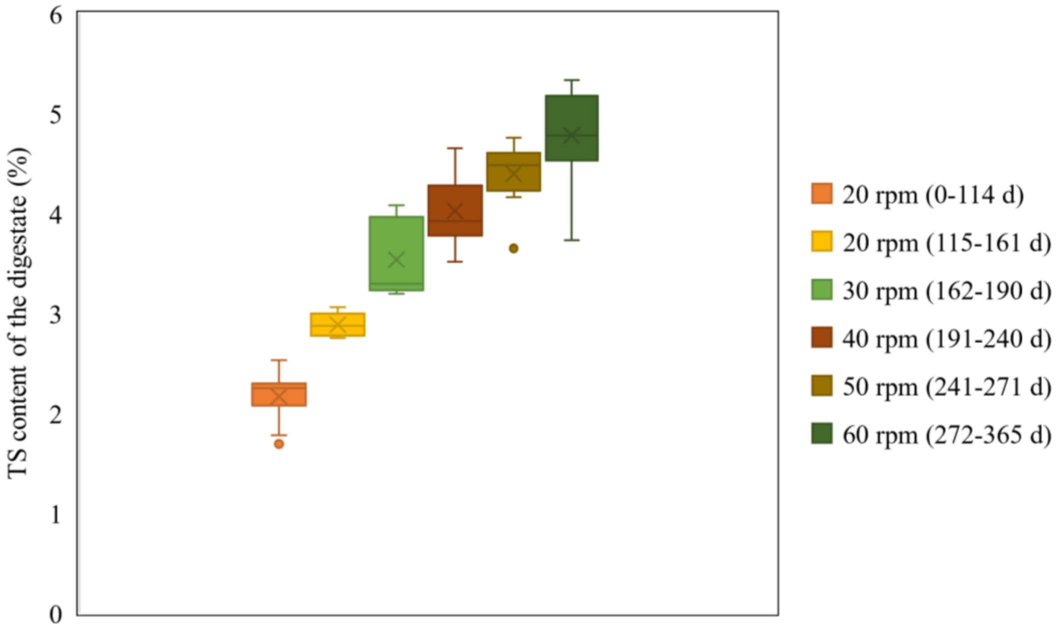
Likewise, at the change to 50 rpm, an increase in biogas production was observed from 351 to 459 $L_{biogas}.kg_{COD}^{-1}$ from day 240 to 248, which corresponded to an increase of 31 %. Afterwards, the production decreased to 379 $L_{biogas}.kg_{COD}^{-1}$ with a minimum value of 344 $L_{biogas}.kg_{COD}^{-1}$. The average biogas production for this period was 382 $L_{biogas}.kg_{COD}^{-1}$, which was upper than the average production during the previous period with a mixing speed of 40 rpm.

The higher mixing speed was 60 rpm. A decrease in biogas production was observed at the change of mixing speed to 60 rpm. The biogas production varied from 379 $L_{biogas}.kg_{COD}^{-1}$ at day 271 to 208 $L_{biogas}.kg_{COD}^{-1}$ at day 320, which was the minimum production observed at this period. Then, the biogas production increased to 440 $L_{biogas}.kg_{COD}^{-1}$ at day 365, with a maximum value of 477 $L_{biogas}.kg_{COD}^{-1}$. This augmentation could be explained by the higher mixing speed used, which allowed a greater contact between the substrate and the microorganisms. The average production was 359 $L_{biogas}.kg_{COD}^{-1}$ with a SD of 58 $L_{biogas}.kg_{COD}^{-1}$. The duration (93 days) for the stabilization of the biogas production was higher at 60 rpm. However, after stabilization, the maximum production exceeded the production previously obtained. The production of biogas was greater with the mixing speed of 60 rpm with a production of half a litter against 0.2 L at previous mixing speed. Biogas yields of 329.2, 410.9, 251.2, 58.3 to 33.7 $L.kg_{VS}^{-1}$ with increase in OLR from 2, 2.5, 3, 3.5 to 4 $g_{VS}.L^{-1}.d^{-1}$ were obtained during the co-digestion of filter cake with sugarcane bagasse in semi-continuously-fed stirred digesters (Leite et al., 2015). Therefore, we obtained higher biogas production than the above study suggesting that intermittent mixing is appropriate for AD of sugarcane vinasse. From these results, we can conclude that the production of biogas increased with the age of the reactor, and that the operating conditions (OLR and intermittent mixing mode) were favorable to the vinasse AD.

A


Biogas production (Lbiogas/kgCOD)
0
50
100
150
200
250
300
350
400
450
500
20 rpm (0-114 d)
20 rpm (115-161 d)
30 rpm (162-190 d)
40 rpm (191-240 d)
50 rpm (241-271 d)
60 rpm (272-365 d)

B


TS content of the digestate (%)
0
1
2
3
4
5
6
20 rpm (0-114 d)
20 rpm (115-161 d)
30 rpm (162-190 d)
40 rpm (191-240 d)
50 rpm (241-271 d)
60 rpm (272-365 d)

C

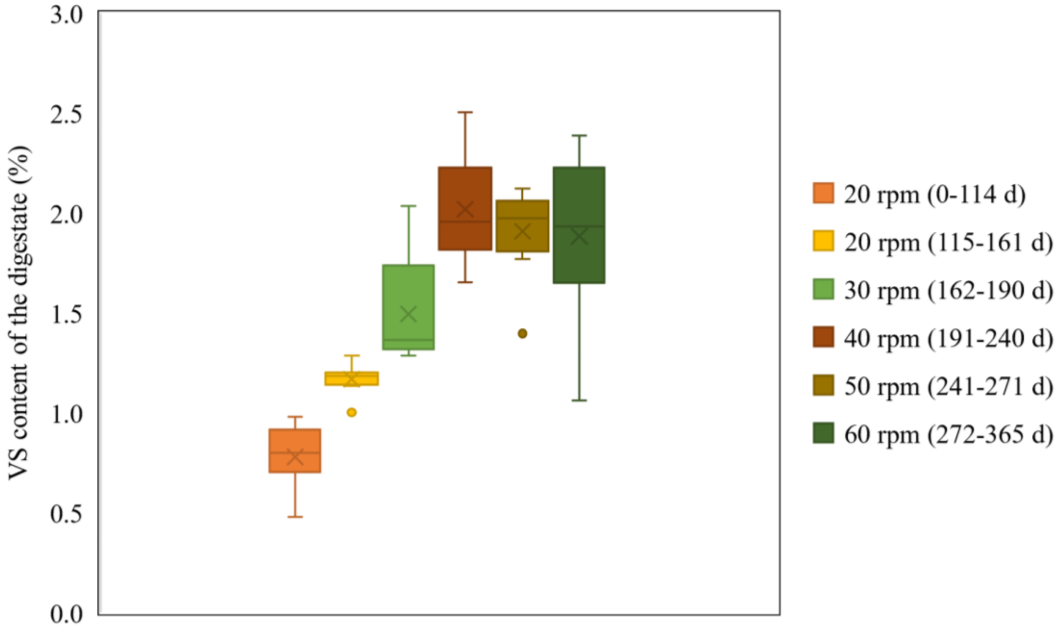
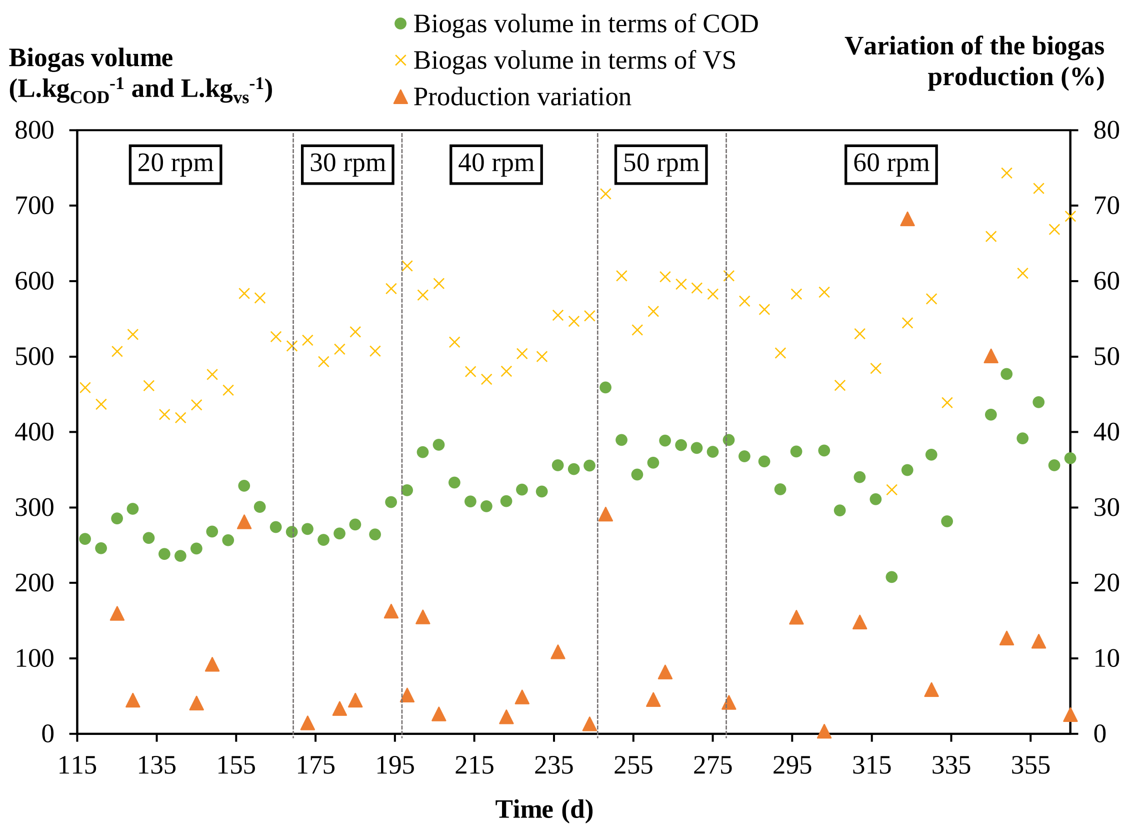


Fig. 2. Boxplot of the biogas production per mixing period (A), boxplot of TS content per mixing period (B), and boxplot of VS content per mixing period (C).

A



B

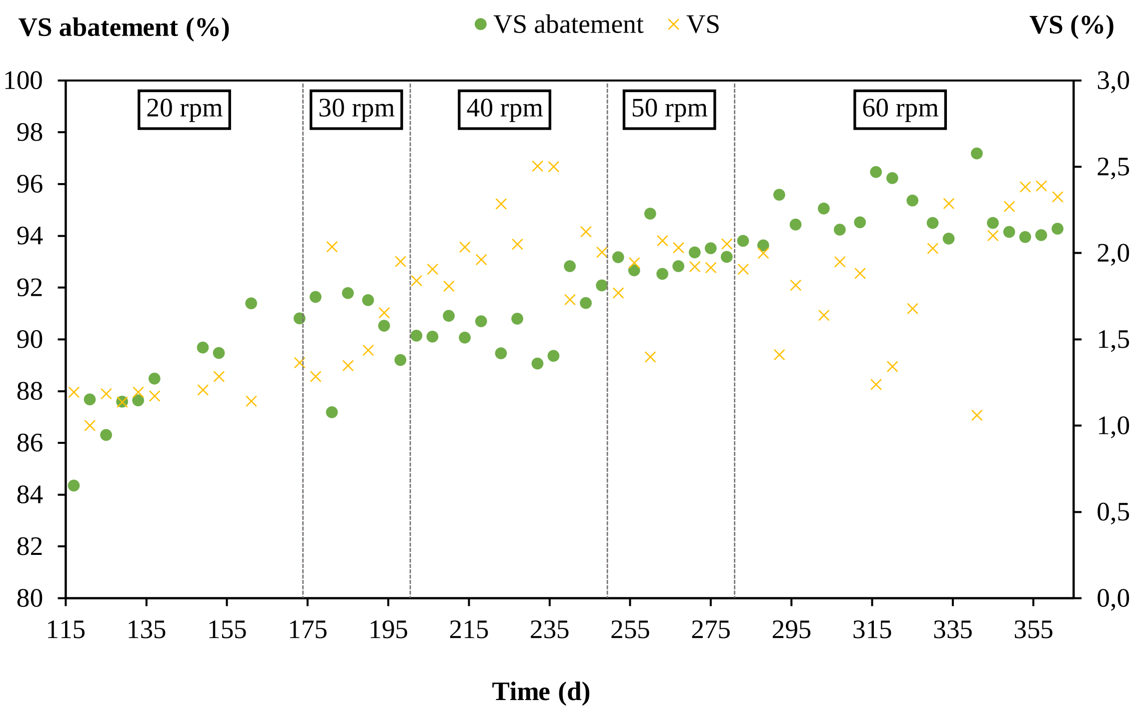


Fig. 3. Biogas production, production variation, and mixing speed (A), and variation of the VS and removal VS during the process (B).

3.3. Study of the effects of physicochemical properties of the digestate on the performances of the digester

3.3.1. VFA and ammonium concentrations

The VFA are the products of the acidogenesis reaction, and the reagents of the acetogenesis. The VFA are composed of acetate, propionate, isobutyrate, butyrate, valerate, and isovalerate (Franke-Whittle et al., 2014) and acetate is the main inhibitory VFA (Xu et al., 2014). The increase in VFA concentration has an impact on the efficient conversion of substrates to biogas (Jackson et al., 2020). Comparing the VFA concentrations of the digestate and the influent, a decrease in VFA concentration was noted. The average VFA concentrations of the vinasse and the digestate were respectively 18.6 ± 3.1 and 7.80 ± 1.69 $g.L^{-1}$. Consequently, the higher VFA concentration in the digestate was due to both the initial VFA concentration of the influent, and the incomplete VFA degradation.

The variation of the VFA and ammonium concentrations are shown on the Fig. 4.A, and the experimental data are provided in supplementary materials. The ammonium concentration was higher and the VFA concentration was lower with the mixing speed of 20 rpm. At the change to 30 rpm, a decrease in ammonium concentration occurred with an increase in VFA concentration. Then, the two concentrations stabilized at 40 rpm. In our study, the VFA concentration was comprised between 8 and 10 $g.L^{-1}$ except at day 341 with a value of 5.48 $g.L^{-1}$. The decrease in VFA concentration to 5.48 $g.L^{-1}$ was followed by an increase in biogas production. Therefore, the decrease in VFA concentration was favorable to the digestion of the vinasse. This decrease in VFA concentration may have been induced by a greater digestion of the hydrolysis products.

The VFA concentration then increased with a slight decrease in biogas production. Therefore, the increase in VFA concentration did not have a negative effect on the biogas production. In addition, the ammonium concentration was inferior to 50 $mg.L^{-1}$ for mixing speed of 40, 50 and 60 rpm. Regarding the biogas production and the variations in ammonium and VFA concentrations, no significant variation of these two concentrations explaining the variations of the biogas production was identified. The biogas production was more important for higher VFA concentrations, and for lower ammonium concentrations.

Janke et al. (2016) compared start-up strategies of AD of sugarcane filter cake co-digested with bagasse. Their work lasted 150 days and included the physicochemical measurements, such as VFA and ammonium concentrations (Janke et al., 2016). The concentration of VFA tended to decrease and reached a value close to zero (Janke et al., 2016). However, they obtained VFA concentration peaks of 2 $g.L^{-1}$ (Janke et al., 2016). Concerning the ammonium concentrations, a similar shape was obtained with a progressive decrease of the concentration, and lower than 500 mg.L^{-1} at the end of the experiment (Janke et al., 2016). The differences between the values of VFA and ammonium concentrations obtained by Janke et al. (2016), and our values can be explained by the differences between the treated substrates. Indeed, our study was carried out with vinasse whereas Janke et al. (2016) studied co-digestion of sugarcane filter cake and bagasse. Souza et al. (1992) studied the thermophilic AD of raw vinasse at pilot scale. During their experiment (260 days), the maximum VFA concentration was approximately 3 g.L^{-1} and the average concentration during the last 50 days of operation was 1.6 g.L^{-1} (Souza et al., 1992). Therefore, their values were also lower to the VFA concentration of the present study.

In conclusion, the experimental measurements of VFA and ammonium concentrations showed that the gradual increase of mixing speed did not impact the biogas production. In addition, the VFA content observed was higher than the VFA content found in literature for similar studies. However, this upper values were not destabilize the AD process. Similar observations were obtained in the case of co-digestion of food waste and cow manure by Franke-Whittle et al. (2014) where the digester operated stably despite high VFA levels (mainly composed of acetate and propionate) and higher pH values (Franke-Whittle et al., 2014). In this work, the optimal ranges of VFA and ammonium concentrations were respectively 8 to 10 g.L^{-1} and 0 to 50 g.L^{-1}. It can be explained by the vinasse used in this study, and the anaerobic digester age. In addition, these higher ranges could be explained by a high proportion of propionate.

3.3.2. Alkalinity and pH

The alkalinity and pH measurements are commonly carried out on biogas plants to detect instabilities. These two parameters are complementary. Indeed, the alkalinity is a measure of the acid-neutralizing capacity, guaranteeing the stability of the pH. An overly alkaline pH could lead to the disintegration of microbial granules and the failure of the process (Franke-Whittle et al., 2014).

The variation of the alkalinity and pH of the digestate are presented on the Fig. 4.B. The pH of the digestate was comprised in the optimum pH range of AD, which is 6.0 to 8.3, as well as the optimum pH range of methanogens, which is 7.0 to 8.0. However, the pH was punctually above 8 for three mixing periods, 40, 50, and 60 rpm.

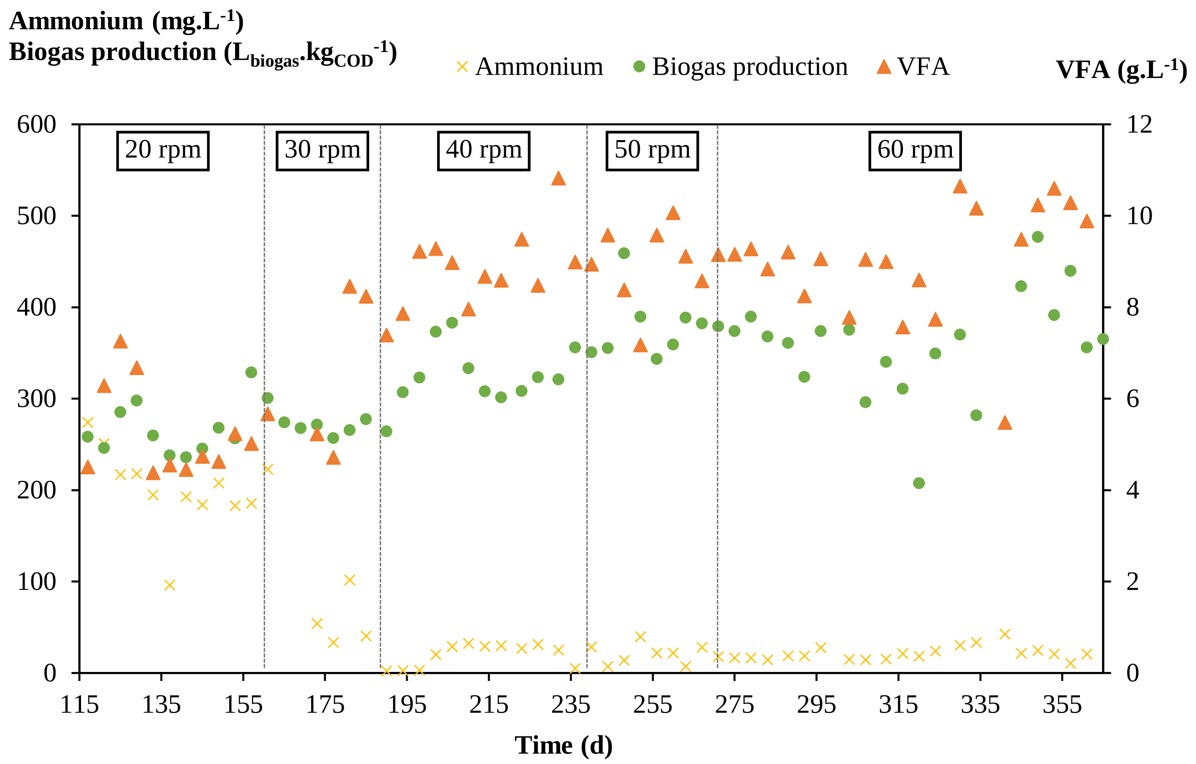
At 20 rpm, the pH slowly decreased from 7.54 to 7.124 and the alkalinity varied from 6.905 to 8.568 g_{CaCO3}.L^{-1}. At the change to 30 rpm, the pH decreased to 6.99 and then increased to 7.70. In parallel, the alkalinity significantly increased to 14.011 g_{CaCO3}.L^{-1}. As a result of the increase in the mixing speed, the pH of the digestate increased, without reaching an inhibitory value for AD. This was consistent with the increase of the alkalinity, which was favorable to the process and limited the pH variations. Indeed, for the rest of the study, the pH stabilized between 7.5 and 8, whereas the alkalinity oscillated between 10.8 and 17.5 $g_{CaCO3}.L^{-1}$ from day 191 to 240. The alkalinity seemed enough to guarantee a stable pH, despite the increase in VFA concentration. A consequence of the increase in pH was an increase in carbonate CO_3^{2-} ions and thus an increase in the chemical capacity of the digestion environment to absorb CO_2. The solubilization of CO_2 was therefore higher as the pH increased. Consequently, the CO_2 content of the biogas was expected to decrease.

The biogas production was higher with greater alkalinity. This result was expected because the alkalinity of the digestion environment was more favorable to AD process. In addition, the increase in VFA concentration led to an increase in pH. The average pH was 7.4 for VFA concentration of 5 $g.L^{-1}$ and the average pH was 7.9 for VFA concentration of 10 $g.L^{-1}$.

The VFA to alkalinity ratio is shown on the Fig. 4.C. The first period corresponded to the start-up of the digester. During this period, the mixing conditions did not vary, and the loading rate was slowly increased. The variations in biogas production were due to the increase in OLR. Likewise, the variations in the VFA / Alk ratio were due to the change in OLR. The maximum ratio was obtained at day 125 with a value of 1.06. This ratio value reflected the VFA accumulation. Furthermore, variations of the ratio were observed at the change in organic loading rate in the digester's start-up phase but also at the change of mixing speed.

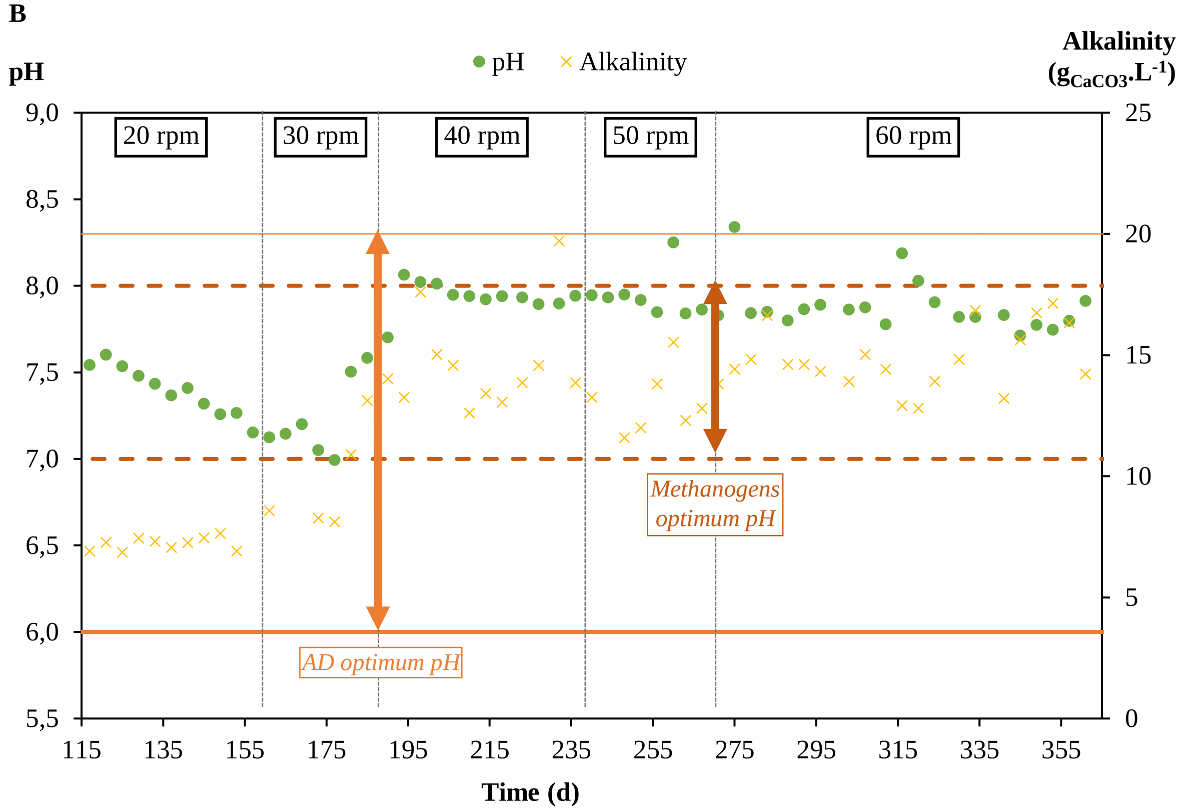
Lossie and Pütz recommended a ratio between 0.3 and 0.4 but still emphasized that this ratio depends on the treated substrate and is therefore specific to each digester (Lossie and Pütz, 2008). The authors recommended a long-term study to determine the optimal ratio for stable operation (Lossie and Pütz, 2008). In our study, the VFA / Alk ratio stabilized at the ratio of 0.5 to 0.7.

A



Ammonium (mg.L^{-1})
Biogas production ($L_{biogas}.kg_{COD}^{-1}$)
Ammonium
Biogas production
VFA
VFA (g.L^{-1})
20 rpm
30 rpm
40 rpm
50 rpm
60 rpm
600
500
400
300
200
100
0
12
10
8
6
4
2
0
115
135
155
175
195
215
235
255
275
295
315
335
355
Time (d)

B



pH
pH
Alkalinity
Alkalinity
($g_{CaCO3}.L^{-1}$)
20 rpm
30 rpm
40 rpm
50 rpm
60 rpm
9,0
8,5
8,0
7,5
7,0
6,5
6,0
5,5
25
20
15
10
5
0
Methanogens optimum pH
AD optimum pH
115
135
155
175
195
215
235
255
275
295
315
335
355
Time (d)

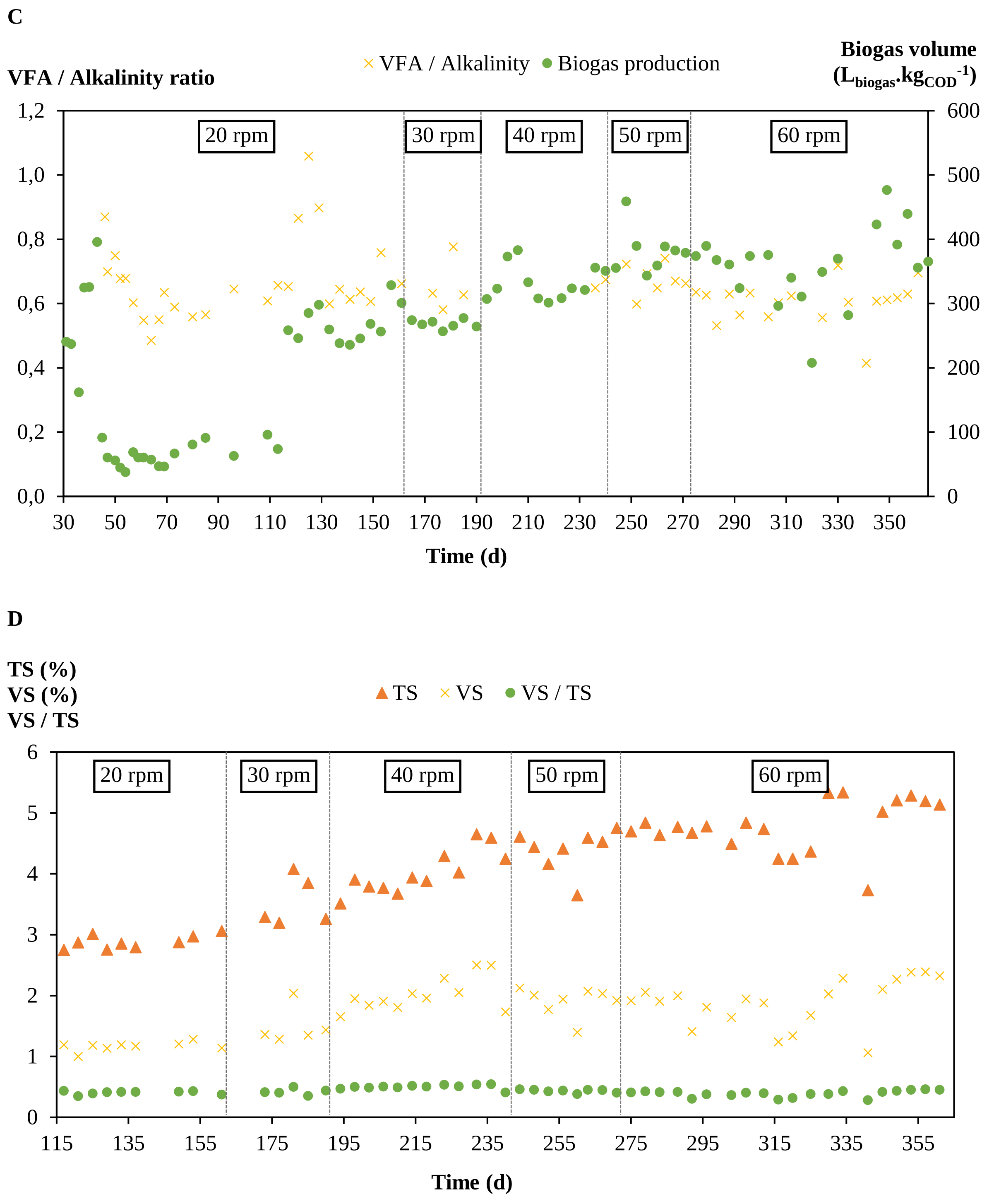


Fig. 4. Evolution of VFA and ammonium concentrations during the process (A), evolution of the alkalinity and pH of the digestate (B), evolution of VFA / Alk ratio during the process (C), and evolution of TS content and VS content during the process (D).

3.4. Study of the local physicochemical properties of TOP, MID, and SLUDGE samples

3.4.1. Alkalinity and pH

The alkalinity and pH before and after mixing of TOP, MID and SLUDGE samples are presented in supplementary material. The sludge had a higher alkalinity than the liquid phase. In addition, the alkalinity of TOP and MID samples was larger after than before mixing. However, the alkalinity remained higher in the mud. The medium presented a stratification in term of alkalinity in the case of vinasse AD submitted to intermittent mixing.

The pH before and after stirring for MID and TOP samples were similar with some exceptions. Indeed, we observed punctual decrease in pH after mixing for both samples between days 200 and 210, and an increase at days 272 and 330. Therefore, the mixing could lead to pH variations. It can be explained by the release of acidic compounds into the environment. However, comparing the pH and the VFA content, no significant variation of the pH was observed. Additional investigations of other physicochemical properties was done in order to identify the impact of mixing on the local properties of the digester.

3.4.2. TS and VS contents

The experimental data of TS and VS contents are given in supplementary material. The Fig. 5.A presents the average TS content per period of TOP, MID and SLUDGE samples before and after mixing. The average variations in TS content per period and per sample before and after mixing is shown on Fig. 5.B. The Fig. 5.C shows the average variations in TS content per period between MID and TOP samples, and MID and SLUDGE samples before and after mixing.

High TS content was observed in the SLUDGE sample. The lowest TS content was reported in TOP sample. According to the Fig. 5.C, the difference in TS content between MID and TOP samples was larger after stirring at 30 and 40 rpm, and lower after stirring at 50 and 60 rpm. Thus, stirring above 50 rpm homogenized the liquid phase of the digester. The increase in the gap is explained by the release of solid matter from the sludge to the liquid phase, resulting in a significant increase in the TS content of the MID sample as shown in Fig. 5.A and Fig. 5.B. Indeed, in Fig. 5.B we observe significant variations in TS content for the MID sample at 30, 40 and 50 rpm compared to the other two samples. Similar variations of VS content occurred. The local study of TS and VS contents showed the effect of mixing on the displacement of solid matters.

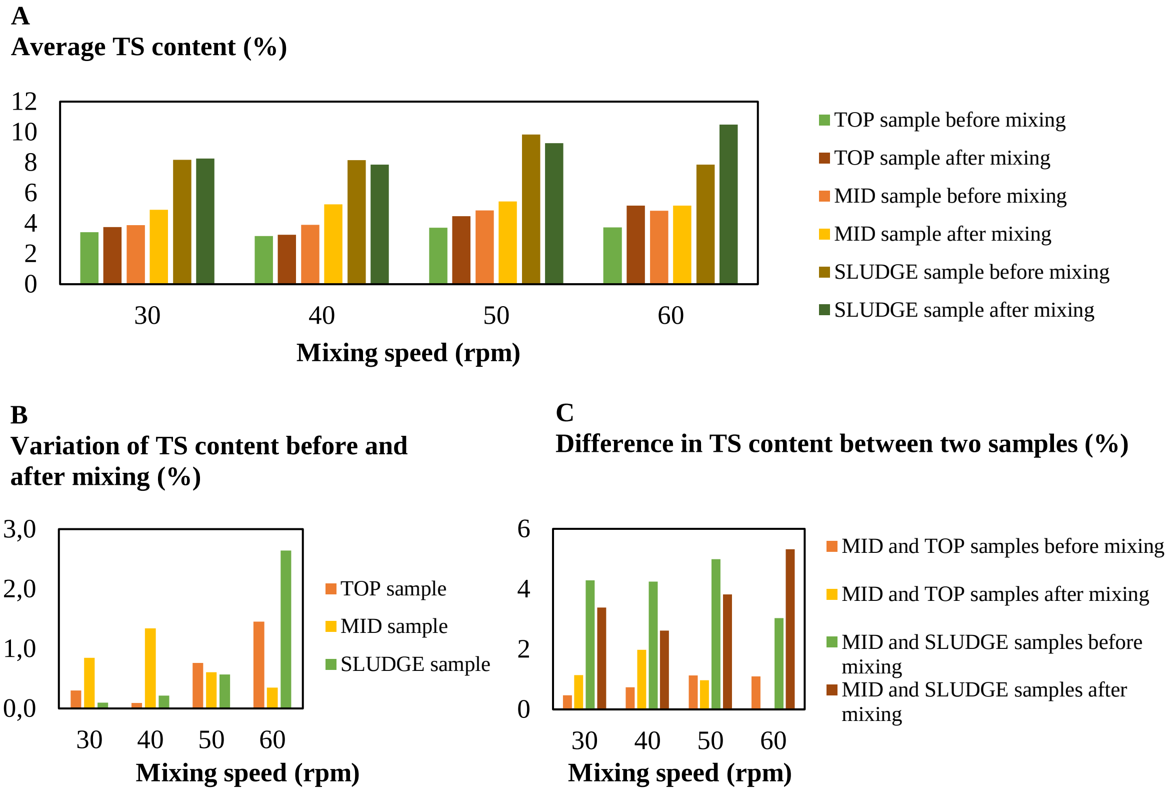


Fig. 5. Average TS content per period of TOP, MID and SLUDGE samples before and after mixing(A), average variations in TS content per period and per sample before and after mixing (B), and average variations in TS content per period between MID and TOP samples, and MID and SLUDGE samples before and after mixing (C).

3.4.3. VFA concentration

The VFA concentration before and after stirring is presented on the Fig. 6.A and the experimental data are provided in supplementary material. An increase in VFA concentration after mechanical agitation for the TOP and MID samples was observed. Indeed, variations of 0.47, 1.06, 1.81 and 4.11 $g.L^{-1}$ for the TOP samples were respectively obtained for the mixing speed of 30, 40, 50 and 60 rpm. The variation in VFA content was larger at 60 rpm. An increase in VFA content of MID samples of 3.82, 2.98, 0.26 and 1.39 $g.L^{-1}$ was observed at 30, 40, 50 and 60 rpm respectively. We can conclude that mixing involved an increase in the VFA concentration in the liquid phase of the digester. In addition, the greater the intensity of agitation, the greater the increase in VFA concentration is marked. These outcomes suggests that the increase in the VFA content is due to the delivery in the liquid phase of the VFAs contained in the sludge. Comparing the three samples, highest VFA content was reached in SLUDGE sample. Therefore, the VFAs have accumulated in the sludge.

The average variations in TS and VS contents per period and per sample before and after mixing are provided on the Fig. 6.B. This graph clearly indicates that the variations in VFA content before and after mixing were higher at 60 rpm for TOP and SLUDGE samples. However, this variation was higher at 30 and 40 rpm for MID sample. Therefore, the lower mixing speeds impacted the VFA concentration in the middle part of the reactor while higher speeds impacted the VFA concentrations in the upper and lower parts.

The biogas production decreased at 60 rpm. This reduction could be explained by the inhibitory effects of VFA. Indeed, the measurements of the digestate showed that the concentration of VFA was stable in the digester. However, the local variations in VFA content were higher at 60 rpm. This result shows the importance of local monitoring of VFA concentrations of anaerobic digesters.

The Fig 6.C exhibits the average variations in VFA concentration per mixing period between MID and TOP samples, and MID and SLUDGE samples before and after mixing. The difference in VFA concentration between the samples SLUDGE and MID was smaller after mixing at 30, 40, 50 and 60 rpm. Conversely, the difference in VFA concentration between MID and TOP samples was higher after mixing at 30 and 40 rpm. However, it was lower at 50 and 60 rpm. Therefore, the four mixing speeds allowed to reduce the heterogeneity in VFA content between the lower parts of the digester (MID and SLUDGE). The mixing speeds of 50 and 60 rpm permitted to homogenize the VFA content the upper parts of the digester (TOP and MID). These measurements also demonstrate the presence of acidic zones within the digester.

Considering the instabilities caused by VFA, precautions should be taken in the case of intermittent mixing as VFA accumulate in the sludge. Indeed, this work showed that the VFA accumulated in the sludge were resuspended in the liquid phase during mixing. This release of VFA into the phase could be the cause of failures.

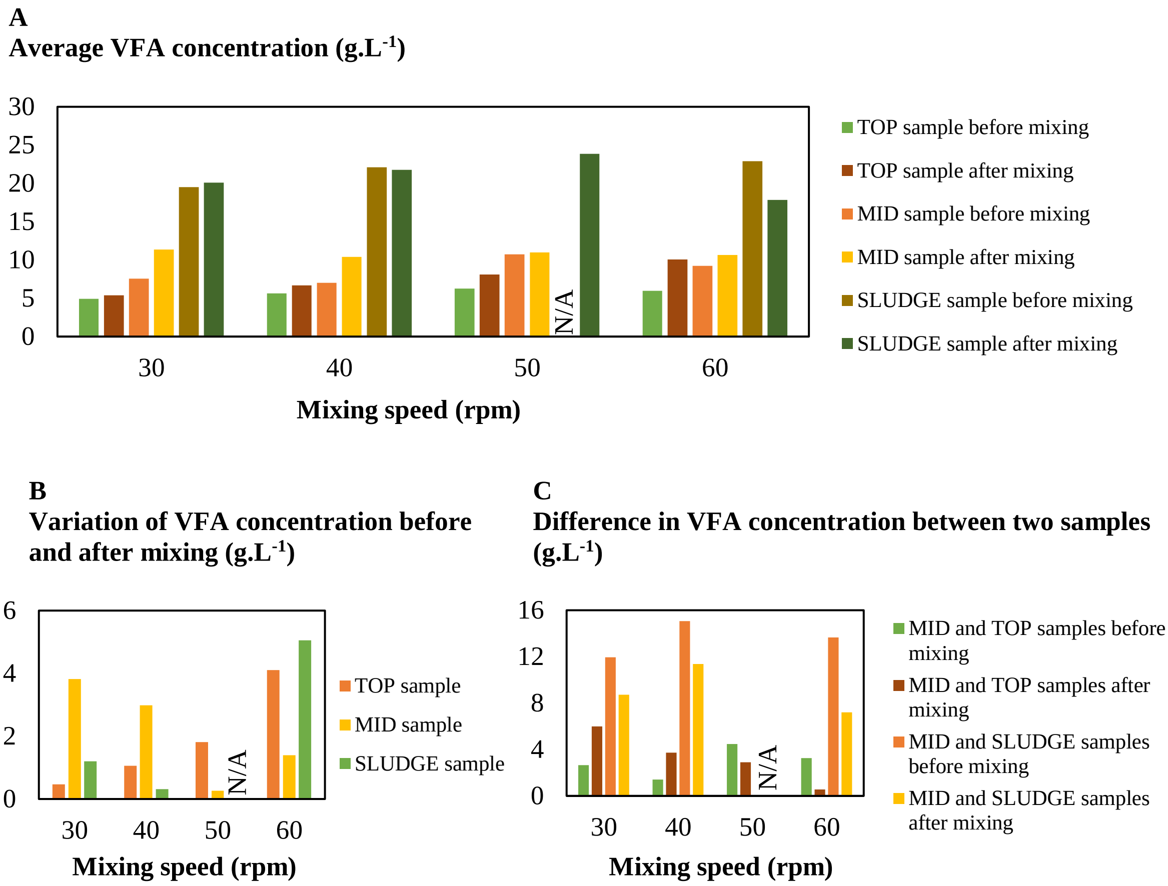


Fig. 6. Average VFA concentrations per period of TOP, MID, and TOP samples before and after mixing (A), average variations in VFA concentrations per period and per sample before and after mixing (B), and average variations in VFA concentrations per period between MID and TOP samples, and MID and SLUDGE samples before and after mixing (C).

4. Conclusions

Vinasse AD with minimal agitation showed high biogas production. Mixing have an impact on the local physicochemical properties of the medium. This local study allowed to identify the stratification of the digestion medium, as well as the acidic zones. This description is useful to detect the inhibitory compounds locally. The local analysis of the medium reveals local physicochemical variations barely visible on the digestate analysis. At the laboratory scale, there are disparities in terms of physicochemical properties depending on the depth of the sample. Therefore, on an industrial scale, this stratification should be even more important.

5. Acknowledgments

This work was supported by the Region Reunion (France) as part of the funding of a research thesis in the PIMENT (Physics and Mathematical Engineering for Energy, Environment and Building) laboratory at the University of Reunion Island. We are also grateful to the Rivière du Mât distillery (Reunion Island) for providing the sugarcane vinasse used in this study.

6. References

Al-Jabari, M., Sawalha, H., Pugazhendhi, A., Rene, E.R., 2021. Cleaner production and resource recovery opportunities in leather tanneries: Technological applications and perspectives. Bioresource Technology Reports 100815. https://doi.org/10.1016/j.biteb.2021.100815

Arimi, M.M., Zhang, Y., Götz, G., Kiriamiti, K., Geißen, S.-U., 2014. Antimicrobial colorants in molasses distillery wastewater and their removal technologies. International Biodeterioration & Biodegradation 87, 34–43. https://doi.org/10.1016/j.ibiod.2013.11.002

Arreola-Vargas, J., Snell-Castro, R., Rojo-Liera, N.M., González-Álvarez, V., Méndez-Acosta, H.O., 2018. Effect of the organic loading rate on the performance and microbial populations during the anaerobic treatment of tequila vinasses in a pilot-scale packed bed reactor: Pilot-scale treatment of tequila vinasses: performance and microbial analysis. Journal of Chemical Technology & Biotechnology 93, 591–599. https://doi.org/10.1002/jctb.5413

Barros, V.G. de, Duda, R.M., Oliveira, R.A. de, 2016. Biomethane production from vinasse in upflow anaerobic sludge blanket reactors inoculated with granular sludge. Brazilian Journal of Microbiology 47, 628–639. https://doi.org/10.1016/j.bjm.2016.04.021

Bollon, J., 2012. Etude des mécanismes physiques et de leur influence sur la cinétique de méthanisation en voie sèche: essais expérimentaux et modélisation. Lyon, INSA.

Caillet, H., Adelard, L., 2020. Start-Up Strategy and Process Performance of Semi-Continuous Anaerobic Digestion of Raw Sugarcane Vinasse. Waste Biomass Valor. https://doi.org/10.1007/s12649-020-00964-z

Caillet, H., Lebon, E., Akinlabi, E., Madyira, D., Adelard, L., 2019. Influence of inoculum to substrate ratio on methane production in Biochemical Methane Potential (BMP) tests of sugarcane distillery waste water. Procedia Manufacturing 35, 259–264. https://doi.org/10.1016/j.promfg.2019.05.037

Cruz-Salomón, A., Ríos-Valdovinos, E., Pola-Albores, F., Meza-Gordillo, R., Lagunas-Rivera, S., Ruíz-Valdiviezo, V.M., 2017. Anaerobic treatment of agro-industrial wastewaters for COD removal in expanded granular sludge bed bioreactor. Biofuel Research Journal 4, 715–720. https://doi.org/10.18331/BRJ2017.4.4.3

Di Maria, F., Sisani, F., Norouzi, O., Mersky, R.L., 2019. The effectiveness of anaerobic digestion of bio-waste in replacing primary energies: An EU28 case study. Renewable and Sustainable Energy Reviews 108, 347–354. https://doi.org/10.1016/j.rser.2019.03.047

Direction de l’Alimentation, de l’Agriculture et de la Forêt de La Réunion, 2016. Présentation de la fillière canne à La Réunion [WWW Document]. Canne. URL http://daaf.reunion.agriculture.gouv.fr/Presentation-production

Franke-Whittle, I.H., Walter, A., Ebner, C., Insam, H., 2014. Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. Waste Management 34, 2080–2089. https://doi.org/10.1016/j.wasman.2014.07.020

Fricke, K., Santen, H., Wallmann, R., Hüttner, A., Dichtl, N., 2007. Operating problems in anaerobic digestion plants resulting from nitrogen in MSW. Waste Management 27, 30–43. https://doi.org/10.1016/j.wasman.2006.03.003

Gosme, M., 2002. Agro-épuration des vinasses de distillerie sur l’île de la Réunion (PhD Thesis). ENSAR.

Jackson, S.A., Kang, X., O’Shea, R., O’Leary, N., Murphy, J.D., Dobson, A.D.W., 2020. Anaerobic digestion performance and microbial community structures in biogas production from whiskey distillers organic by-products. Bioresource Technology Reports 12, 100565. https://doi.org/10.1016/j.biteb.2020.100565

Jain, M.S., Kalamdhad, A.S., 2018. A review on management of Hydrilla verticillata and its utilization as potential nitrogen-rich biomass for compost or biogas production. Bioresource Technology Reports 1, 69–78. https://doi.org/10.1016/j.biteb.2018.03.001

Janke, L., Leite, A., Nikolausz, M., Schmidt, T., Liebetrau, J., Nelles, M., Stinner, W., 2015. Biogas Production from Sugarcane Waste: Assessment on Kinetic Challenges for Process Designing. International Journal of Molecular Sciences 16, 20685–20703. https://doi.org/10.3390/ijms160920685

Janke, L., Leite, A.F., Nikolausz, M., Radetski, C.M., Nelles, M., Stinner, W., 2016. Comparison of start-up strategies and process performance during semi-continuous anaerobic digestion of sugarcane filter cake co-digested with bagasse. Waste Management 48, 199–208. https://doi.org/10.1016/j.wasman.2015.11.007

Kaparaju, P., Buendia, I., Ellegaard, L., Angelidakia, I., 2008. Effects of mixing on methane production during thermophilic anaerobic digestion of manure: Lab-scale and pilot-scale studies. Bioresource Technology 99, 4919–4928. https://doi.org/10.1016/j.biortech.2007.09.015

Kariyama, I.D., 2018. Influence of mixing on anaerobic digestion efficiency in stirred tank digesters: A review. Water Research 15.

Khalil, M., Berawi, M.A., Heryanto, R., Rizalie, A., 2019. Waste to energy technology: The potential of sustainable biogas production from animal waste in Indonesia. Renewable and Sustainable Energy Reviews 105, 323–331. https://doi.org/10.1016/j.rser.2019.02.011

Leite, A., Janke, L., Lv, Z., Harms, H., Richnow, H.-H., Nikolausz, M., 2015. Improved Monitoring of Semi-Continuous Anaerobic Digestion of Sugarcane Waste: Effects of Increasing Organic Loading Rate on Methanogenic Community Dynamics. IJMS 16, 23210–23226. https://doi.org/10.3390/ijms161023210

Lindmark, J., Thorin, E., Bel Fdhila, R., Dahlquist, E., 2014. Effects of mixing on the result of anaerobic digestion: Review. Renewable and Sustainable Energy Reviews 40, 1030–1047. https://doi.org/10.1016/j.rser.2014.07.182

López González, L.M., Pereda Reyes, I., Romero Romero, O., 2017. Anaerobic co-digestion of sugarcane press mud with vinasse on methane yield. Waste Management 68, 139–145. https://doi.org/10.1016/j.wasman.2017.07.016

Lossie, U., Pütz, P., 2008. Targeted control of biogas plants with the help of FOS/TAC. Practice Report Hach-Lange.

Mohammadrezaei, R., Zareei, S., Behroozi- Khazaei, N., 2018. Optimum mixing rate in biogas reactors: Energy balance calculations and computational fluid dynamics simulation. Energy. https://doi.org/10.1016/j.energy.2018.06.132

Moraes, B.S., Triolo, J.M., Lecona, V.P., Zaiat, M., Sommer, S.G., 2015. Biogas production within the bioethanol production chain: Use of co-substrates for anaerobic digestion of sugar beet vinasse. Bioresource Technology 190, 227–234. https://doi.org/10.1016/j.biortech.2015.04.089

Nery, V.D., 2018. Hydraulic and organic rates applied to pilot scale UASB reactor for sugar cane vinasse degradation and biogas generation. Biomass and Bioenergy 7.

Paulose, P., Kaparaju, P., 2021. Anaerobic mono-digestion of sugarcane trash and bagasse with and without pretreatment. Industrial Crops and Products 170, 113712. https://doi.org/10.1016/j.indcrop.2021.113712

Ramos-Vaquerizo, F., Cruz-Salomón, A., Ríos-Valdovinos, E., Pola-Albores, F., Lagunas-Rivera, S., 2018. Anaerobic Treatment of Vinasse from Sugarcane Ethanol Production in Expanded Granular Sludge Bed Bioreactor. Journal of Chemical Engineering and Process Technology 9, 3.

Sahota, S., Shah, G., Ghosh, P., Kapoor, R., Sengupta, S., Singh, P., Vijay, V., Sahay, A., Vijay, V.K., Thakur, I.S., 2018. Review of trends in biogas upgradation technologies and future perspectives. Bioresource Technology Reports 1, 79–88. https://doi.org/10.1016/j.biteb.2018.01.002

Singh, B., Szamosi, Z., Siménfalvi, Z., 2019. State of the art on mixing in an anaerobic digester: A review. Renewable Energy 141, 922–936. https://doi.org/10.1016/j.renene.2019.04.072

Souza, M.E., Fuzaro, G., Polegato, A.R., 1992. Thermophilic Anaerobic Digestion of Vinasse in Pilot Plant UASB Reactor. Water Science and Technology 25, 213–222. https://doi.org/10.2166/wst.1992.0153

Sulaiman, A., Hassan, M.A., Shirai, Y., Abd-Aziz, S., Tabatabaei, M., Busu, Z., Yacob, S., 2009. The Effect of Mixing on Methane Production in a Semi-commercial Closed Digester Tank Treating Palm Oil Mill Effluent 8.

Sun, Q., Li, H., Yan, J., Liu, L., Yu, Z., Yu, X., 2015. Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. Renewable and Sustainable Energy Reviews 51, 521–532. https://doi.org/10.1016/j.rser.2015.06.029

Van Hulle, S.W.H., Vesvikar, M., Poutiainen, H., Nopens, I., 2014. Importance of scale and hydrodynamics for modeling anaerobic digester performance. Chemical Engineering Journal 255, 71–77. https://doi.org/10.1016/j.cej.2014.06.041

Vavilin, V.A., Angelidaki, I., 2005. Anaerobic degradation of solid material: Importance of initiation centers for methanogenesis, mixing intensity, and 2D distributed model. Biotechnology and Bioengineering 89, 113–122. https://doi.org/10.1002/bit.20323

Vavilin, V.A., Lokshina, L.Y., Flotats, X., Angelidaki, I., 2007. Anaerobic digestion of solid material: Multidimensional modeling of continuous-flow reactor with non-uniform influent concentration distributions. Biotechnology and Bioengineering 97, 354–366. https://doi.org/10.1002/bit.21239

Wiedemann, L., Conti, F., Saidi, A., Sonnleitner, M., Goldbrunner, M., 2018. Modeling Mixing in Anaerobic Digesters with Computational Fluid Dynamics Validated by Experiments. Chemical Engineering & Technology 41, 2101–2110. https://doi.org/10.1002/ceat.201800083

Xu, Z., Zhao, M., Miao, H., Huang, Z., Gao, S., Ruan, W., 2014. In situ volatile fatty acids influence biogas generation from kitchen wastes by anaerobic digestion. Bioresource Technology 163, 186–192. https://doi.org/10.1016/j.biortech.2014.04.037

Appendices: supplementary data

Appendix A: Experimental data of the influent

Table A.1. Feeding rate and influent concentration.

Samples and collection date	Vinasse-1 08-2018	Vinasse-2 10-2018	Vinasse-3 12-2018	Average ± SD
Period (d)	0-156	157-197	198-365	-
Volume added / removed (L)	1.5	1.5	1.5	1.5 ± 0
Feeding rate ($L.d^{-1}$)	0.375	0.375	0.375	0.375 ± 0
OLR ($g_{VS}.L^{-1}.d^{-1}$)	1.27	1.31	1.51	1.36 ± 0.13
OLR ($g_{COD}.L^{-1}.d^{-1}$)	2.26	2.52	2.35	2.38 ± 0.13
Variation in terms of VS (%) when changing vinasse feedstock	-	+3.15	-15.27	-
Variation in terms of COD (%) when changing vinasse feedstock	-	+11.50	-6.75	-

Table A.2. Physicochemical characteristics of vinasse.

Characteristics	Inoculum	Vinasse-1 (Caillet and Adelard, 2020)	Vinasse-2	Vinasse-3	Average value ± SD
Period (days)		0-156	157-197	198-365	-
BMP ($NL_{CH4}.kg_{COD}^{-1}$)	-	185.59	185.59	185.59	185.59
TS %	3.67	7.09	7.97	8.50	7.85 ± 0.71
VS ($\%_{sample}$)	2.21	4.88	5.04	5.78	5.23 ± 0.48
pH	-	4.84	-	4.63	4.74 ± 0.15
COD $g_{O2}.L^{-1}$	37.20	86.70	96.80	90.10	91.2 ± 5.14
TOC $mg.L^{-1}$	12,670	29,875	30,900	-	30,388 ± 725
VFA $mg.L^{-1}$	8,390	19,360	15,237	21,340	18,646 ± 3
Am $mg.L^{-1}$	45.53	37.40	44.93	33.90	38.74 ± 5.64
Alk $mg_{CaCO3}.L^{-1}$	9,107	1,080.86	2,007	2,302	1,796 ± 637
N $mg.L^{-1}$	2,185	1,120.00	1,333	-	1,227 ± 151
Alk / COD	0.25	0.01	0.02	0.03	0.02 ± 0.01
VFA / Alk	0.92	17.91	7.59	9.27	11.59 ± 5.54
C / N ratio	5.85	26.67	23.18	-	24.93 ± 2.47

Appendix B: Results on the biogas production

Table B.1. Minimum, maximum, and average biogas production ($L_{biogas}.kg_{COD}^{-1}$) per mixing period.

Period	Mixing speed (rpm)	Duration (days)	Minimum production	Maximum production	A1* ± SD	A2* ± SD
0-114 (Caillet and Adelard, 2020)	20	114	38	396	121 ± 106	-
115-161	20	46	236	329	268 ± 29	270 ± 32
162-190	30	28	257	278	268 ± 7	266 ± 9
191-240	40	49	302	383	332 ± 27	332 ±27
241-271	50	30	344	459	382 ± 35	371 ± 19
272-365	60	93	208	477	359 ± 58	355 ± 63

A1*: Average production; A2*: Average production without the first three values.

Table B.2. Average methane production ($L_{CH4}.kg_{COD}^{-1}$) in function of the proportion of methane.

Mixing speed (rpm)	Average methane production in function of proportion of methane				
	65 %	60 %	55 %	50 %	45 %
20	174.2	160.8	147.4	134.0	120.6
30	174.2	160.8	147.4	134.0	120.6
40	**215.8**	**199.2**	182.6	166.0	149.4
50	**248.3**	**229.2**	**210.1**	**191.0**	171.9
60	**233.4**	**215.4**	**197.5**	179.5	161.6

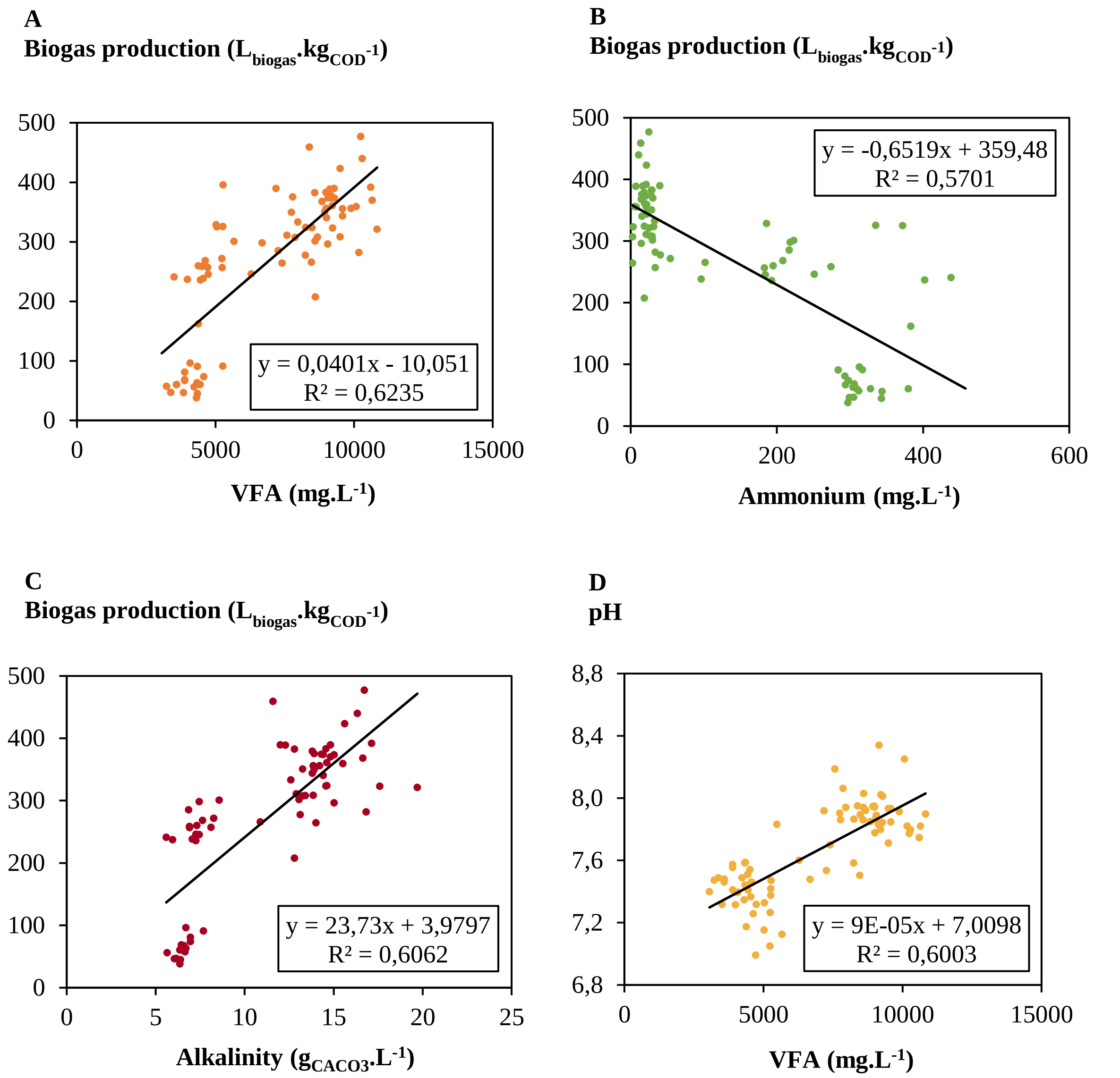


Fig. B.1. Biogas production in function of VFA concentration (A), biogas production in function of ammonium concentration (B), biogas production in function of alkalinity (C) and pH in function of VFA concentration (D).

Appendix C: Experimental data on the physicochemical properties of the samples TOP, MID and SLUDGE

Table C.1. VFA concentration ($g.L^{-1}$).

Days	Before agitation			After agitation			Variation		
	TOP	MID	SLUDGE	TOP	MID	SLUDGE	TOP	MID	SLUDGE
30 rpm (161-190 days)									
A*	4.92	7.56	19.50	5.39	11.38	20.10	0.47	3.82	-1.20
SD**	0.60	-	-	0.40	-	2.83	0.33	-	5.37
40 rpm (190-240 days)									
A*	5.63	7.03	22.09	6.69	10.42	21.78	1.06	2.98	-0.31
SD**	0.60	2.82	10.35	0.45	1.46	6.81	0.66	2.66	13.16
50 rpm (240-271 days)									
A*	6.28	10.74	N/A	8.09	10.99	23.85	1.81	0.26	N/A
SD**	1.14	3.23	-	1.45	1.21	-	2.22	4.02	-
60 rpm (271-365 days)									
A*	5.98	9.25	22.90	10.08	10.64	17.85	4.11	1.39	-5.05
SD**	0.39	3.64	-	0.07	0.16	-	0.46	3.48	-

*Average value, **Standard deviation

Table C.2. Alkalinity concentration ($g_{CaCO3}.L^{-1}$).

Days	Before agitation			After agitation			Variation		
	TOP	MID	SLUDGE	TOP	MID	SLUDGE	TOP	MID	SLUDGE
30 rpm (161-190 days)									
A*	8.59	11.69	18.88	8.89	17.04	19.94	0.3	5.35	3.75
SD**	0.91	-	1.14	0.45	-	4.66	0.45	-	0.45
40 rpm (190-240 days)									
A*	12.19	14.97	30.82	12.83	16.77	28.89	1.32	1.89	-1.93
SD**	1.95	2.47	17.94	1.66	2.21	5.63	1.40	2.30	22.10
50 rpm (240-271 days)									
A*	11.14	13.81	27.47	13.18	14.14	22.88	2.04	0.33	-4.59
SD**	0.54	1.74	-	1.39	0.91	-	1.09	2.11	-
60 rpm (271-365 days)									
A*	11.95	14.72	11.09	14.22	14.87	34.52	2.27	0.15	23.43
SD**	0.36	0.57	-	2.14	0.35	-	1.78	0.22	-

*Average value, **Standard deviation

Table C.3. TS content (%).

Days	Before agitation			After agitation			Variation		
	TOP	MID	SLUDGE	TOP	MID	SLUDGE	TOP	MID	SLUDGE
30 rpm (161-190 days)									
A*	3.17	3.9	8.15	3.26	5.24	7.86	0.09	1.34	0.81
SD**	0.22	-	0.41	0.28	-	0.93	0.06	-	0.84
40 rpm (190-240 days)									
A*	3.42	3.88	8.17	3.75	4.88	8.27	0.30	0.85	0.10
SD**	0.13	0.73	1.29	0.24	0.82	1.10	0.14	0.73	0.77
50 rpm (240-271 days)									
A*	3.72	4.84	9.84	4.48	5.48	9.27	0.76	0.61	-0.57
SD**	0.12	0.53	-	0.34	0.29	-	0.30	0.64	-
60 rpm (271-365 days)									
A*	3.73	4.83	7.86	5.188	5.18	10.50	1.45	0.35	2.64
SD**	0.02	1.12	-	0.05	0.37	-	0.03	0.75	-

*Average value, **Standard deviation

Table C.4. VS content (%).

Days	Before agitation			After agitation			Variation		
	TOP	MID	SLUDGE	TOP	MID	SLUDGE	TOP	MID	SLUDGE
30 rpm (161-190 days)									
A*	1.09	1.74	5.31	1.25	1.35	5.02	0.16	-0.39	0.08
SD**	0.34	-	0.52	0.15	-	0.63	0.19	-	0.51
40 rpm (190-240 days)									
A*	1.06	1.15	4.66	1.44	2.31	4.82	0.34	1.06	0.16
SD**	0.15	0.56	0.53	0.20	0.65	0.58	0.11	0.52	0.64
50 rpm (240-271 days)									
A*	1.37	2.25	6.04	1.98	2.91	5.61	0.61	0.66	-0.43
SD**	0.08	0.44	-	0.24	0.19	-	0.23	0.25	-
60 rpm (271-365 days)									
A*	1.17	1.98	4.35	1.81	2.16	6.17	0.64	0.19	1.82
SD**	0.30	0.52	-	0.81	0.49	-	1.10	0.02	-

*Average value, **Standard deviation

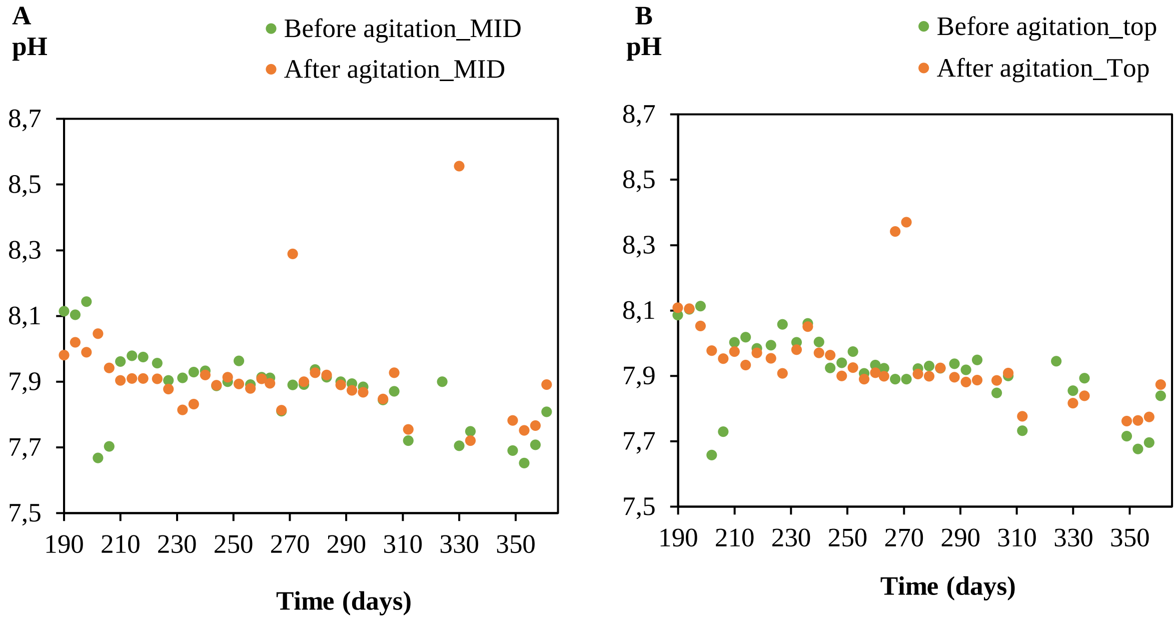


Fig. C.1. pH measurements before and after agitation of MID sample (A) and TOP sample (B) at 40 rpm (191-240 days), 50 rpm (241-271 days), and 60 rpm (272-365 days).

Table C.6. Maximum, minimum and average values of physicochemical analysis of the effluent at different mixing speeds.

Parameters	Units	115-161 days			162-190 days			191-240 days		
		20 rpm			30 rpm			40 rpm		
		Min	Max	A*	Min	Max	A*	Min	Max	A*
Ammonium	$mg.L^{-1}$	96.3	274.0	202.36	2.5	102.0	46.6	2.6	32.6	22.0
VFA	$g.L^{-1}$	4.38	7.26	5.29	4.72	8.46	6.81	7.87	10.83	8.94
Alkalinity	$g_{CaCO3}.L^{-1}$	6.85	8.57	7.33	8.11	14.01	10.88	12.60	19.70	14.56
VFA / Alk	-	0.60	1.06	0.73	0.58	0.78	0.65	0.65	0.67	0.66
pH	-	7.12	7.60	7.37	6.99	7.70	7.31	7.89	8.06	7.95
TS	%	2.75	3.06	2.88	3.19	4.08	3.53	3.51	4.65	4.02
VS	%	1.00	1.28	1.17	1.29	2.04	1.49	1.65	2.50	2.02
VS / TS	-	0.35	0.43	0.41	0.35	0.50	0.42	0.41	0.54	0.50

Parameters	Units	241-271 days			272-365 days			Average		
		50 rpm			60 rpm					
		Min	Max	A*	Min	Max	A*	Min	Max	A*
Ammonium	$g.L^{-1}$	7.2	39.9	19.8	10.9	42.5	21.4	23.9	98.2	64.4
VFA	$g.L^{-1}$	7.18	10.07	8.96	5.48	10.65	9.02	5.93	9.45	7.80
Alkalinity	$g_{CaCO3}.L^{-1}$	11.59	15.52	13.12	13.20	17.14	15.09	10.47	14.99	12.2
VFA / Alk	-	0.60	0.74	0.68	0.42	0.72	0.60	0.57	0.79	0.66
pH	-	7.83	8.25	7.93	7.71	8.34	7.88	7.51	7.99	7.69
TS	%	3.65	4.61	4.34	3.73	5.34	4.78	3.37	4.35	3.91
VS	%	1.40	2.12	1.91	1.06	2.39	1.88	1.28	2.07	1.69
VS / TS	-	0.38	0.46	0.44	0.28	0.46	0.39	0.35	0.48	0.43

*Average value