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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. *Bioresource Technology Reports*, 2021, 16, pp.100837. 10.1016/j.biteb.2021.100837 . hal-03727620

**HAL Id: hal-03727620**

**<https://hal.science/hal-03727620>**

Submitted on 21 Jul 2022

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# 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

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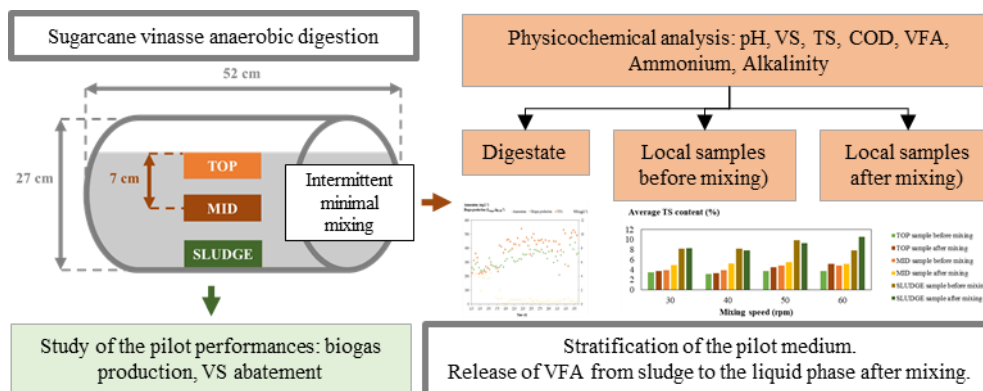
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## Abstract

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  $477 \text{ L}_{\text{CH}_4} \cdot \text{kg}_{\text{COD}}^{-1}$  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  $10 \text{ g} \cdot \text{L}^{-1}$ . 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



21 **Keywords**

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

24 **Highlights**

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

30 **Statement of novelty**

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

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

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

39

## 40 **1. Introduction**

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

48 Reunion Island is a French department localized in the Indian Ocean (55° 32' E, 21° 08' S), in a tropical  
49 environment. The sugarcane culture is predominant in this region of 2,512 km<sup>2</sup>. According to the DAAF  
50 (Directorate of Food, Agriculture and Forestry), the surface dedicated to sugarcane corresponds approximately to  
51 52.94 % of the agricultural area (Direction de l'Alimentation, de l'Agriculture et de la Forêt de La Réunion,  
52 2016). In 2015, about 1,896,656 tons of sugarcane were produced, which allowed the production of 201,181 tons  
53 of sugar, 80,000 HAP (hectoliters of pure alcohol) and 513,000 tons of bagasse (Direction de l'Alimentation, de  
54 l'Agriculture et de la Forêt de La Réunion, 2016). The alcohol is produced from the molasses, resulting from the  
55 refining of sugar extracted from sugar beet or sugarcane. In Reunion Island, the molasses are used for the  
56 production of rum, 38 % to the Savanna distillery, 59 % to the Rivière du Mât distillery and 3 % to the Isautier  
57 distillery (Gosme, 2002). In 2000, approximately 150,000 tons of the vinasses were produced (Gosme, 2002).  
58 The rejection of vinasses may cause environmental pollution such as salinity, phytotoxicity, anoxia,  
59 eutrophication, death of aquatic life, and many severe health problems due to its high pollutant content (Cruz-  
60 Salomón et al., 2017; Ramos-Vaquerizo et al., 2018). Anaerobic digestion (AD) is an appropriate method to  
61 reduce the pollutant content of this organic waste while producing biogas (Al-Jabari et al., 2021; Di Maria et al.,  
62 2019). This natural process is based on the degradation of organic matter under the action of micro-organisms in  
63 the absence of oxygen (Jain and Kalamdhad, 2018). The biogas produced, rich in methane and carbon dioxide, is  
64 a source of renewable energy (Khalil et al., 2019; Sun et al., 2015). The biogas has several applications,  
65 including fuel production, the substitution of the natural gas or electricity generation (Sahota et al., 2018).  
66 Ramos-Vaquerizo et al. (2018) underlined that this treatment is the most environmentally beneficial technology  
67 and energy-efficient production.

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

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

88 Mixing generates energy consumption, directly linked to operating costs. Indeed, up to 50 % of the energy  
89 consumption in biogas plant is due to mixing (Wiedemann et al., 2018). The mode of agitation has a major  
90 impact on the reduction of electricity consumption. Minimal mixing consists in stirring prior to  
91 extraction/feeding phase, and intermittent agitation is any discontinuous agitation. In this context, minimal  
92 mixing allows to reduce the energy demand and maintenance cost and to improve the biogas production  
93 (Kariyama, 2018). Kaparaju et al. (2008) compared minimal mixing (mixing for 10 min prior to  
94 extraction/feeding), intermittent mixing (withholding mixing for 2 h prior to extraction/feeding) and continuous  
95 feeding, with three slurry anaerobic digesters. Minimal mixing is a method to homogenize the medium rather  
96 than mixing. The highest methane production was obtained with minimal mixing (Kaparaju et al., 2008). This  
97 type of mixing is thus promising. Experimental study carried out by Sulaiman et al. (2009) allowed to conclude

98 that the minimal mixing is sufficient to permit good contact between the substrate and the microorganisms, as  
99 well as to release the entrapped biogas at the bottom of the digester. In addition, the impact of mixing should be  
100 studied on various substrates, and mixing modes, as the proper mixing depends on the nature of the substrate  
101 (Lindmark et al., 2014). The results obtained on the slurry cannot be applied to the vinasse, and no work has  
102 been done on AD of vinasse with minimal intermittent mixing. Van Hulle *et al.* showed that at pilot scale  
103 (120 L) the methane production, due to VFA accumulation, was impacted by the mixing or non-mixing  
104 conditions (Van Hulle et al., 2014). Therefore, the analysis of the physicochemical properties of the digestate  
105 could contribute to the understanding of the effect of mixing on the process.

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

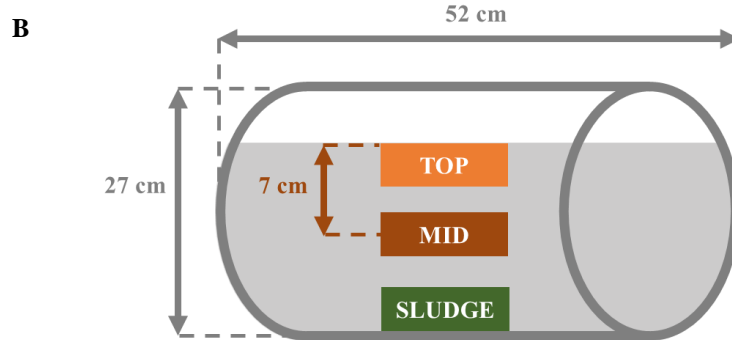
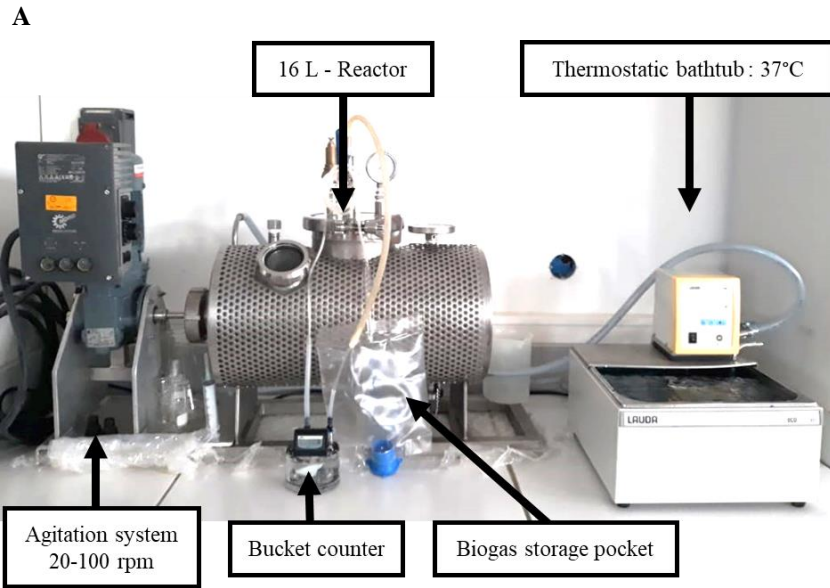
## 111 **2. Material and Methods**

### 112 **2.1. Digestion process and digester**

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

117 The mechanical stirring could be set from 20 to 100 rpm with a step of 10 rpm. The impeller consisted in two  
118 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  
119 sheet of 0.001 m thickness.

120 The digester was equipped with a security safety valve and a dial for monitoring the temperature of the digestion  
121 medium. The bucket counter was used to measure the biogas volume produced with a measuring chamber  
122 volume of 3.18 mL, a minimum flow of 1 mL.h<sup>-1</sup> and a maximum flow of 1 L.h<sup>-1</sup>. The set-up is described on the  
123 Fig. 1.A.



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

125

## 126 **2.2. Substrate, inoculum, and physicochemical analysis**

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

135 Prior to this study, the 16-L digester has already been running for 130 days, and the inoculum was already  
136 acclimatized to the sugarcane vinasse from the distillery Rivière du Mât (Reunion Island). The experimental data  
137 for this period were presented in a previous work (Caillet and Adelard, 2020) and emphasized in the results  
138 section. The start-up of the digester and the increase of the organic load were detailed in (Caillet and Adelard,  
139 2020). The same feedstock was used in this work. The substrate and the inoculum were homogenized at  
140 12,000 rpm for 10 min before the characterization tests. The Total Solid (TS) content was obtained after drying  
141 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  
142 at 550 °C. The VFA, the ammonium, the Chemical Oxygen Demand (COD), the Total Organic Carbon (TOC),  
143 the Nitrogen (N), and the alkalinity of the substrate were measured on the input. The chemical tests were  
144 conducted using the Hach Lange tests. The biochemical methane potential (BMP) test was carried out in a  
145 previous work and the results are provided in supplementary material (Caillet et al., 2019; Caillet and Adelard,  
146 2020).

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

152 The removed VS content was calculated as follows:

$$153 \quad VS_{\text{Removal}} = \frac{VS_{\text{digested}}}{VS_{\text{added}}} * 100 = \frac{VS_{\text{added}} - VS_{\text{measured}}}{VS_{\text{added}}} * 100 \quad (1)$$



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

### 155 **2.3. Organic Loading, HRT, and mixing conditions**

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

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

$$159 \quad HRT = \frac{WV}{Q} \quad (2)$$

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

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

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

### 169 **2.4. Statistical analysis**

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

175 The SD was calculated as follows:

$$176 \quad \sigma = \sqrt{\frac{1}{N} \sum_{i=1}^n (x_i - \bar{x})^2} \quad (3)$$

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

## 178 **3. Results and Discussion**

179 **3.1. Substrate and inoculum characteristics**

180 The physicochemical properties of the inoculum and the vinasse used are given in supplementary material. The  
181 average TS and VS contents of the vinasse are respectively  $7.85 \pm 0.71$  % and  $5.23 \pm 0.48$  %. The vinasse is  
182 characterized by an acidic pH with a value of  $4.74 \pm 0.15$ . The average COD, TOC, VFA, ammonium, and  
183 alkalinity are  $91.2 \pm 5.14$  g<sub>O2</sub>.L<sup>-1</sup>,  $30,388 \pm 725$  mg.L<sup>-1</sup>,  $18,646 \pm 3$  VFA mg.L<sup>-1</sup>,  $38.74 \pm 5.64$  mg.L<sup>-1</sup>, and  
184  $1,796 \pm 637$  mg<sub>CaCO3</sub>.L<sup>-1</sup>.

185 The alkalinity (Alk), TOC, COD, ammonium and nitrogen concentrations and the TS and VS content of the  
186 vinasse-2 are upper to those of the vinasse-1. Nevertheless, the VFA concentration of the vinasse-2 is lower to  
187 those of the vinasse-1. The Alk / COD and C / N ratio of the vinasse samples are close with an average value of  
188  $0.02 \pm 0.01$  and  $24.93 \pm 2.47$ . C / N ratio comprised between 20 and 30 was considered in literature as the  
189 optimum ratio for AD process because sufficient nitrogen is available for microbial growth and the organic  
190 carbon can be degraded (Fricke et al., 2007). The VFA / Alk ratio is more variable with 17.9 for the vinasse-1,  
191 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  
192 VFA concentration. In addition, the vinasse-2 has the highest value of COD and ammonium concentration.  
193 Globally, the chemical properties of the three vinasse feedstocks are close, but the analysis showed some  
194 variations, mostly at the level of VFA concentration and alkalinity with a SD of 3,114 mg.L<sup>-1</sup> and 637 mg<sub>CaCO3</sub>.L<sup>-1</sup>  
195 respectively.

196 The OLR as a function of the sample of vinasse used was studied. The vinasse-1, vinasse-2, and vinasse-3  
197 correspond to the vinasse taken from the Rivière du Mât distillery recovered at intervals of 2 months. The  
198 variations of VS content and COD were calculated between the used sample and the previous substrate sample.  
199 The variation of OLR in terms of VS content was negligible (3.15 %), whereas the variation in terms of COD  
200 was higher (11.50 %) between vinasse-1 to the vinasse-2 samples. Concerning the vinasse-3, the OLR in terms  
201 of VS and COD varied respectively by 15.27 and 6.75 %. The average values of OLR in terms of VS and COD  
202 were respectively  $1.36 \pm 0.13$  g<sub>VS</sub>.L<sup>-1</sup>.d<sup>-1</sup> and  $2.38 \pm 0.13$  g<sub>COD</sub>.L<sup>-1</sup>.d<sup>-1</sup> with the SD which respectively represented  
203 9.55 and 5.46 % of the average value. In conclusion, the OLR varied non-significantly. The feeding rate and  
204 influent concentration of each samples are given in supplementary material.

205 **3.2. Study of the process performance of the digester**

206 The minimum, maximum, median, SD and average biogas production per mixing period are presented in the  
207 Fig. 2.A. The boxplots of the TS content and VS content of the digestate for each mixing period are respectively

208 presented in Fig. 2.B and Fig. 2.C. The Fig. 3.A shows the biogas production at different mixing speeds and the  
209 variation in biogas production between feeding as a percentage of production variation. Each point corresponds  
210 to the production obtained for 1500 mL of vinasse added in the digester. The variations of the VS content and  
211 removal VS are shown on the Fig. 3.B. The experimental data are given in supplementary material.

212 The removal VS was upper than 80 % for the whole study with a maximum value of 97.18 % (Fig. 3.B). Both  
213 the removal VS and the VS content gradually increased along the process. The TS solid content increased in  
214 function of the time whereas the VS content stabilized from the mixing period of 40 rpm. The dispersion was  
215 higher for the VS content than the TS content (Fig. 2.B and Fig. 2.C). The average TS content of the sludge and  
216 the vinasse were respectively 3.67 and  $7.85 \pm 0.71$  %. The TS content variation of the digestate varied from 0.31,  
217 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  
218 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  
219 VS contents increased linearly during the process despite the mixing changes. The TS and VS content  
220 respectively increased by 0.01 and 0.005 % per day. This augmentation reflected the accumulation of the solid  
221 matters in the digester.

222 The proportion of methane in biogas for AD of vinasse in mesophilic conditions is 65 % (Arreola-Vargas et al.,  
223 2018; Nery, 2018). Considering this percentage, the methane production is presented in supplementary material  
224 for different proportions of methane, from 65 to 45 % of methane. The BMP of the vinasse was  
225  $185.59 \text{ NL}_{\text{CH}_4} \cdot \text{kgCOD}^{-1}$ . Therefore, the methane production cannot exceed this value. The maximum proportion of  
226 methane was 65 % at 20 rpm and 30 rpm, 55 % at 40 rpm, 45 % at 50 rpm, and 50 % at 60 rpm.

227 The first period (0-114 d) corresponds to the start-up of the reactor. The study of the start-up of the digester was  
228 presented in a previous work (Caillet and Adelard, 2020). During this period, the mixing speed was 20 rpm. An  
229 important data dispersion on this period was reported with a SD of  $106 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  (Fig. 2.A). This important  
230 dispersion is logical because this period corresponds to the start-up period of the digester exhibiting significant  
231 variations in biogas production. In fact, start-up of anaerobic digester is recognized as a critical phase with  
232 important risks of unbalance and failure of the process (Paulose and Kaparaju, 2021). Significant dispersions of  
233 biogas production were also observed in the periods 2 (115-161 d) and 6 (272-365 d), corresponding respectively  
234 to mixing speeds of 20 and 60 rpm. This results suggest that the biogas production was less stable during these  
235 periods. The variations of biogas production in period 2 can be explained by the age of the anaerobic reactor  
236 during this period (Bollon, 2012). In fact, the acclimation of microorganisms provides the reactor with resistance

237 to high VFA concentrations (Bollon, 2012). The instabilities in biogas production observed at period 6 could be  
238 explained by the higher mixing speed applied during the period 6. The largest dispersions of the TS and VS  
239 contents of the digestate were also observed in period 6 (Fig. 2.B and Fig. 2.C). It can be concluded that the  
240 digestion process was less stable at the period 6.

241 The average biogas production with the mixing speed of 20 rpm and 30 rpm were  $268 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  with a SD  
242 of 29 and  $7 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  respectively. No remarkable variation in biogas production was observed at mixing  
243 speed change from 20 to 30 rpm (day 161).

244 An increase in biogas production was noted at mixing speed change from 30 to 40 rpm (day 190). Indeed, the  
245 biogas production ranged from 264 to  $383 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  from day 190 to 206, which represented an increase of  
246 45 %. Then, the production slowly decreased, but remained higher than the production obtained for the mixing  
247 speed of 30 rpm. This punctual increase in biogas production could be due to the return of undigested material  
248 accumulated at the bottom of the digester in the liquid phase, or to the evacuation of the gas bubbles under the  
249 action of the agitation. The average biogas production for this period was  $332 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$ . The average  
250 production with an agitation of 40 rpm was higher than the production with lower agitation.

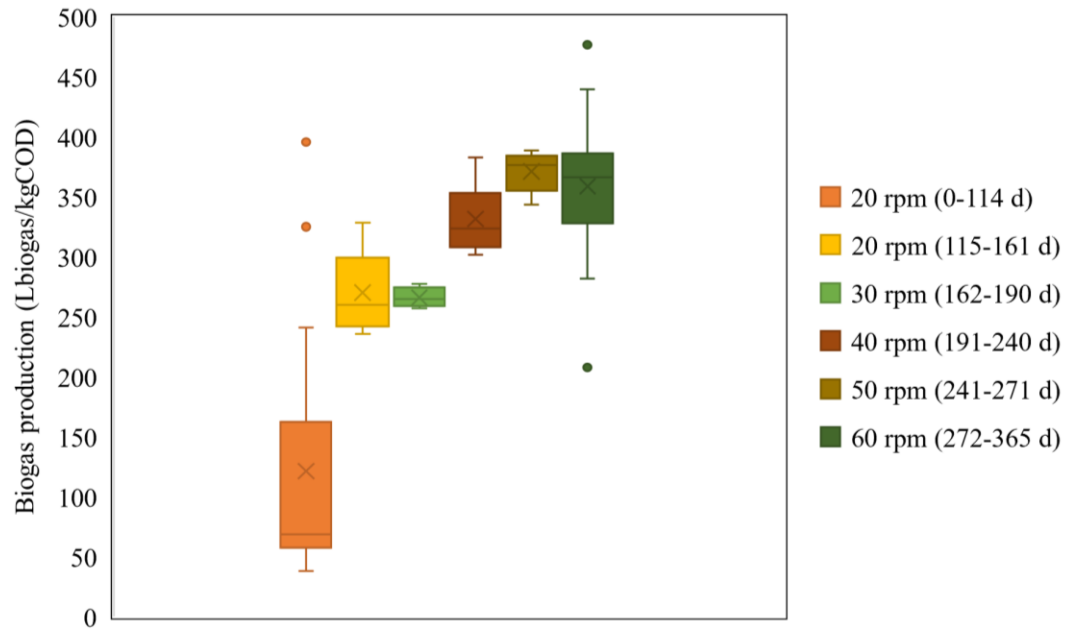
251 Likewise, at the change to 50 rpm, an increase in biogas production was observed from 351 to  $459 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$   
252 from day 240 to 248, which corresponded to an increase of 31 %. Afterwards, the production decreased to  
253  $379 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  with a minimum value of  $344 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$ . The average biogas production for this period  
254 was  $382 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$ , which was upper than the average production during the previous period with a mixing  
255 speed of 40 rpm.

256 The higher mixing speed was 60 rpm. A decrease in biogas production was observed at the change of mixing  
257 speed to 60 rpm. The biogas production varied from  $379 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  at day 271 to  $208 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  at day  
258 320, which was the minimum production observed at this period. Then, the biogas production increased to  
259  $440 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  at day 365, with a maximum value of  $477 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$ . This augmentation could be  
260 explained by the higher mixing speed used, which allowed a greater contact between the substrate and the  
261 microorganisms. The average production was  $359 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$  with a SD of  $58 \text{ L}_{\text{biogas}} \cdot \text{kgCOD}^{-1}$ . The duration (93  
262 days) for the stabilization of the biogas production was higher at 60 rpm. However, after stabilization, the  
263 maximum production exceeded the production previously obtained. The production of biogas was greater with  
264 the mixing speed of 60 rpm with a production of half a litter against 0.2 L at previous mixing speed. Biogas  
265 yields of 329.2, 410.9, 251.2, 58.3 to  $33.7 \text{ L} \cdot \text{kgVS}^{-1}$  with increase in OLR from 2, 2.5, 3, 3.5 to  $4 \text{ g}_{\text{VS}} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$  were

266 obtained during the co-digestion of filter cake with sugarcane bagasse in semi-continuously-fed stirred digesters  
267 (Leite et al., 2015). Therefore, we obtained higher biogas production than the above study suggesting that  
268 intermittent mixing is appropriate for AD of sugarcane vinasse. From these results, we can conclude that the  
269 production of biogas increased with the age of the reactor, and that the operating conditions (OLR and  
270 intermittent mixing mode) were favorable to the vinasse AD.

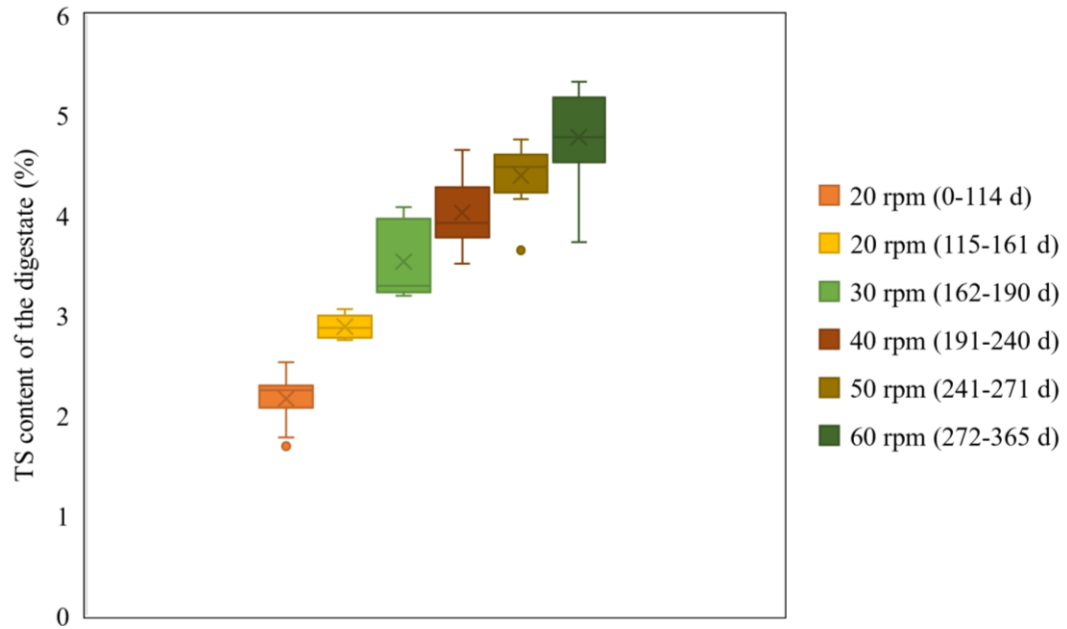
271

**A**

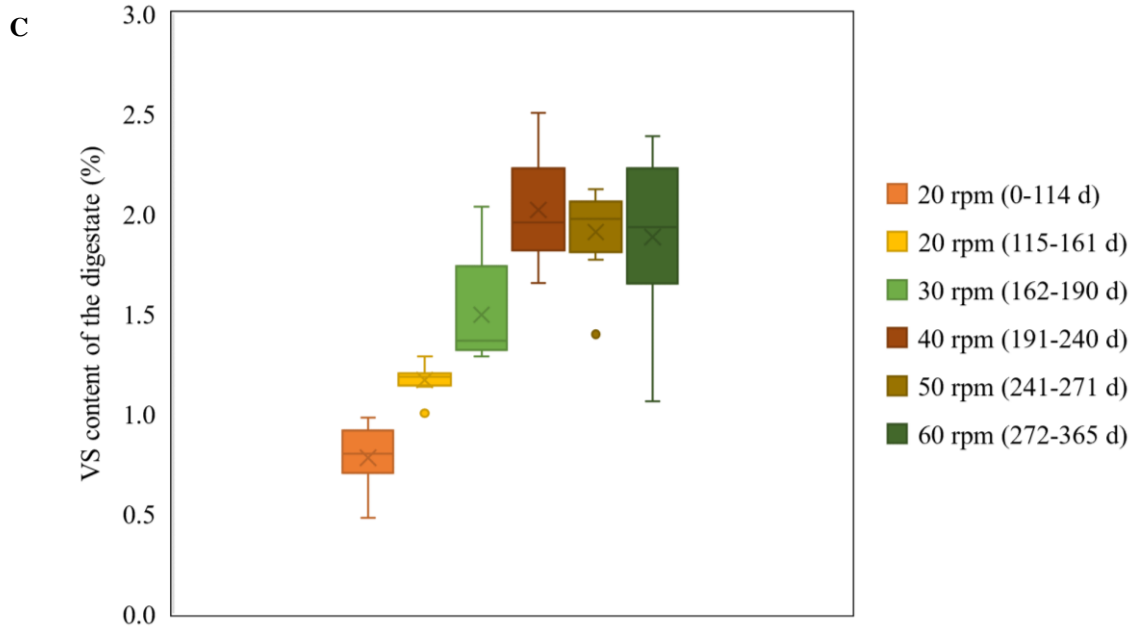


272

**B**



273

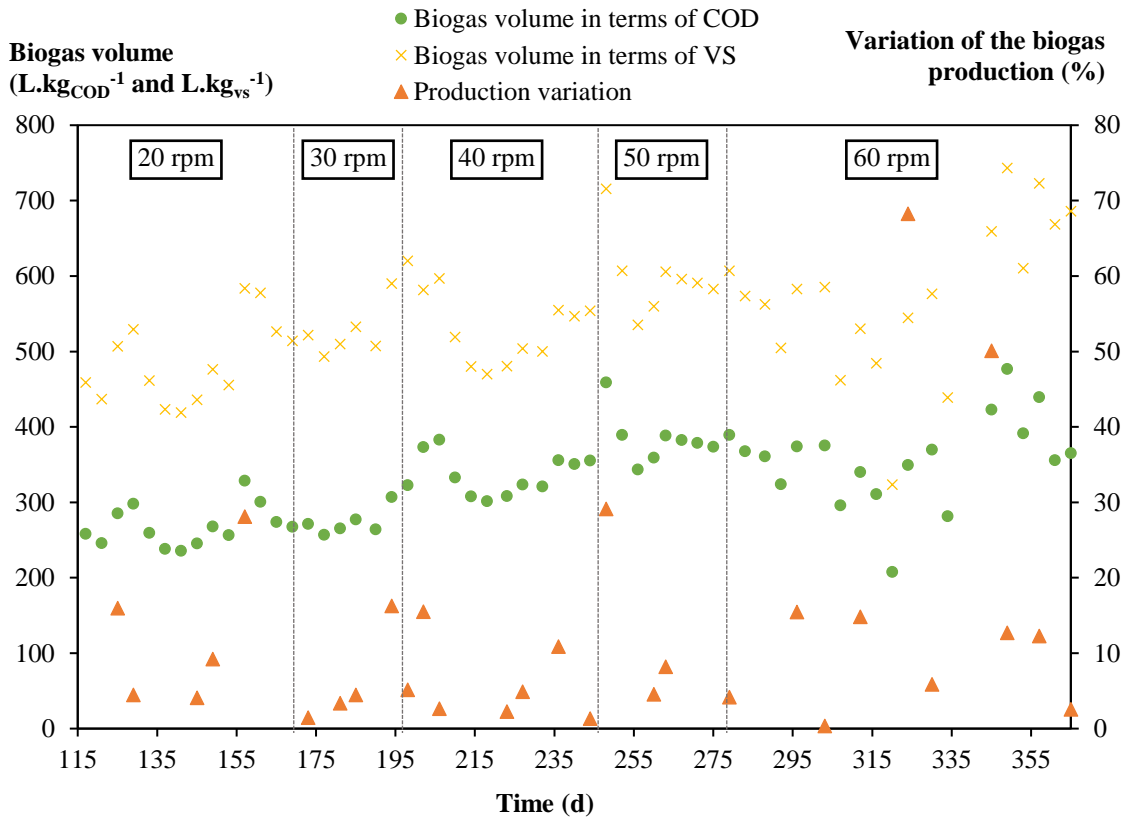


274

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

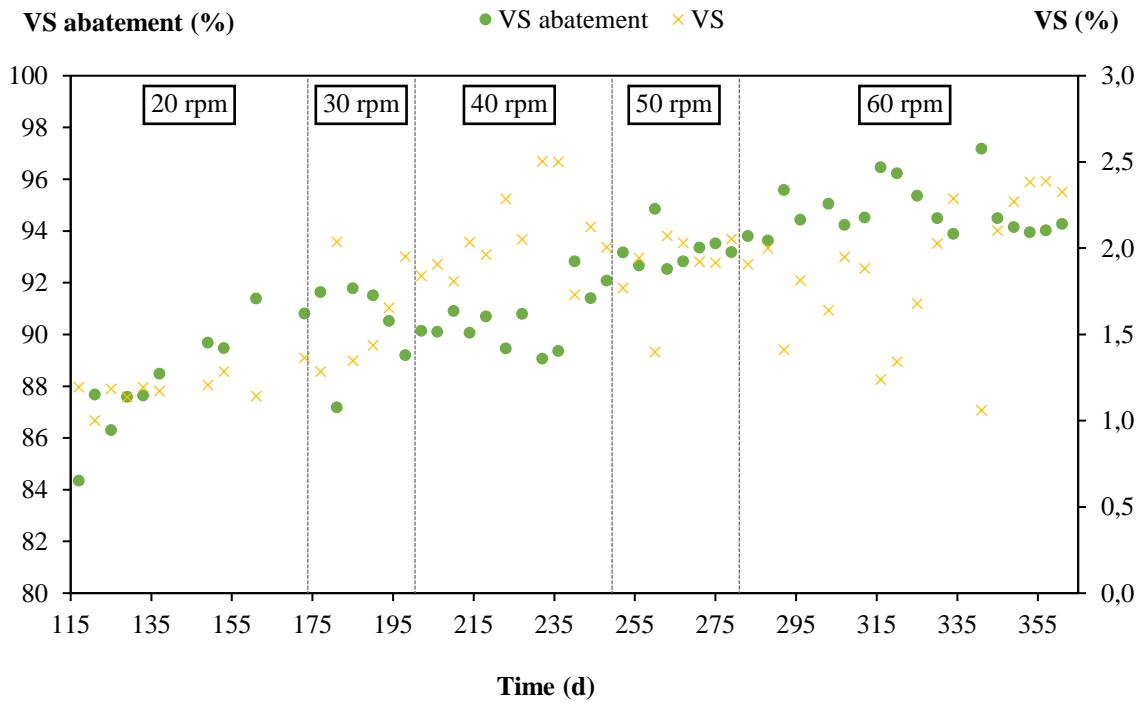
276 boxplot of VS content per mixing period (C).

A



277

B



278

279 **Fig. 3.** Biogas production, production variation, and mixing speed (A), and variation of the VS and removal VS

280 during the process (B).



### 281 **3.3. Study of the effects of physicochemical properties of the digestate on the performances of the digester**

#### 282 **3.3.1. VFA and ammonium concentrations**

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

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

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

305 Janke et al. (2016) compared start-up strategies of AD of sugarcane filter cake co-digested with bagasse. Their  
306 work lasted 150 days and included the physicochemical measurements, such as VFA and ammonium  
307 concentrations (Janke et al., 2016). The concentration of VFA tended to decrease and reached a value close to  
308 zero (Janke et al., 2016). However, they obtained VFA concentration peaks of 2 g.L<sup>-1</sup> (Janke et al., 2016).  
309 Concerning the ammonium concentrations, a similar shape was obtained with a progressive decrease of the

310 concentration, and lower than 500 mg.L<sup>-1</sup> at the end of the experiment (Janke et al., 2016). The differences  
311 between the values of VFA and ammonium concentrations obtained by Janke et al. (2016), and our values can be  
312 explained by the differences between the treated substrates. Indeed, our study was carried out with vinasse  
313 whereas Janke et al. (2016) studied co-digestion of sugarcane filter cake and bagasse. Souza et al. (1992) studied  
314 the thermophilic AD of raw vinasse at pilot scale. During their experiment (260 days), the maximum VFA  
315 concentration was approximately 3 g.L<sup>-1</sup> and the average concentration during the last 50 days of operation was  
316 1.6 g.L<sup>-1</sup> (Souza et al., 1992). Therefore, their values were also lower to the VFA concentration of the present  
317 study.

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

### 327 **3.3.2. Alkalinity and pH**

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

332 The variation of the alkalinity and pH of the digestate are presented on the Fig. 4.B. The pH of the digestate was  
333 comprised in the optimum pH range of AD, which is 6.0 to 8.3, as well as the optimum pH range of  
334 methanogens, which is 7.0 to 8.0. However, the pH was punctually above 8 for three mixing periods, 40, 50, and  
335 60 rpm.

336 At 20 rpm, the pH slowly decreased from 7.54 to 7.124 and the alkalinity varied from 6.905 to 8.568 g<sub>CaCO3</sub>.L<sup>-1</sup>.

337 At the change to 30 rpm, the pH decreased to 6.99 and then increased to 7.70. In parallel, the alkalinity  
338 significantly increased to 14.011 g<sub>CaCO3</sub>.L<sup>-1</sup>. As a result of the increase in the mixing speed, the pH of the

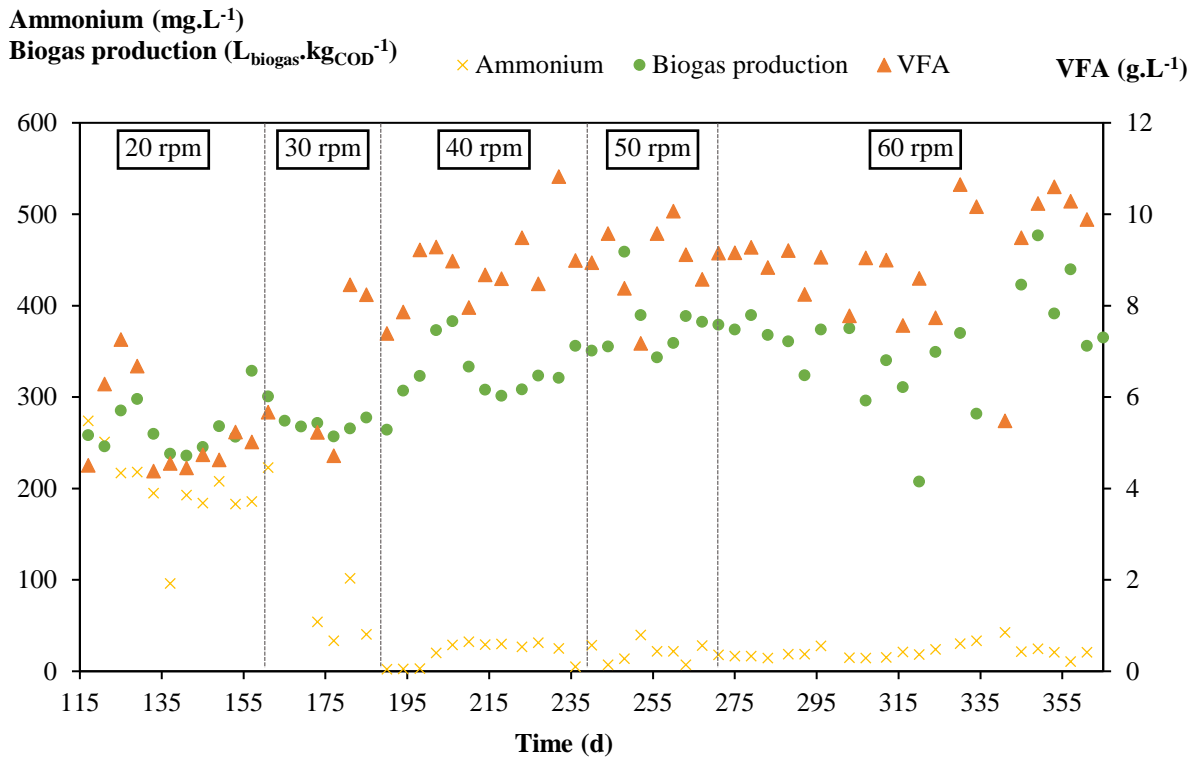
339 digestate increased, without reaching an inhibitory value for AD. This was consistent with the increase of the  
340 alkalinity, which was favorable to the process and limited the pH variations. Indeed, for the rest of the study, the  
341 pH stabilized between 7.5 and 8, whereas the alkalinity oscillated between 10.8 and 17.5  $\text{g}_{\text{CaCO}_3}\cdot\text{L}^{-1}$  from day 191  
342 to 240. The alkalinity seemed enough to guarantee a stable pH, despite the increase in VFA concentration. A  
343 consequence of the increase in pH was an increase in carbonate  $\text{CO}_3^{2-}$  ions and thus an increase in the chemical  
344 capacity of the digestion environment to absorb  $\text{CO}_2$ . The solubilization of  $\text{CO}_2$  was therefore higher as the pH  
345 increased. Consequently, the  $\text{CO}_2$  content of the biogas was expected to decrease.

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

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

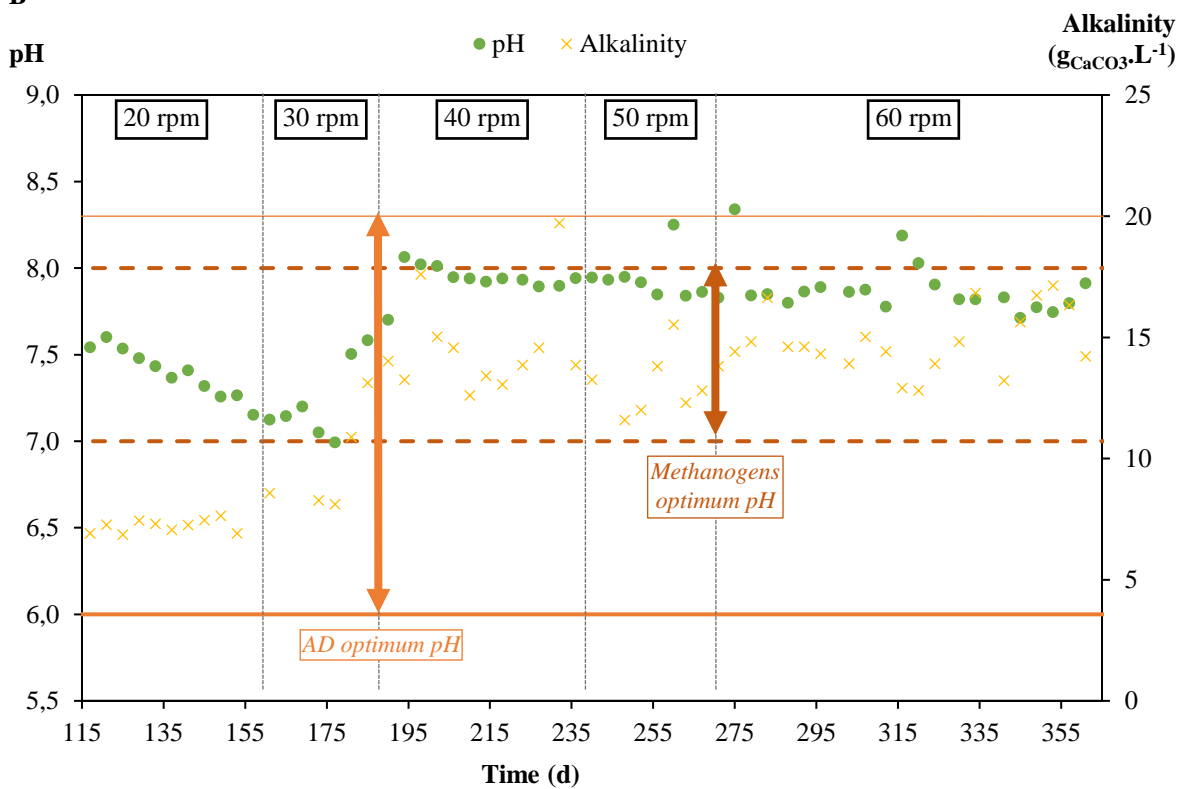
356 Lossie and Pütz recommended a ratio between 0.3 and 0.4 but still emphasized that this ratio depends on the  
357 treated substrate and is therefore specific to each digester (Lossie and Pütz, 2008). The authors recommended a  
358 long-term study to determine the optimal ratio for stable operation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

A



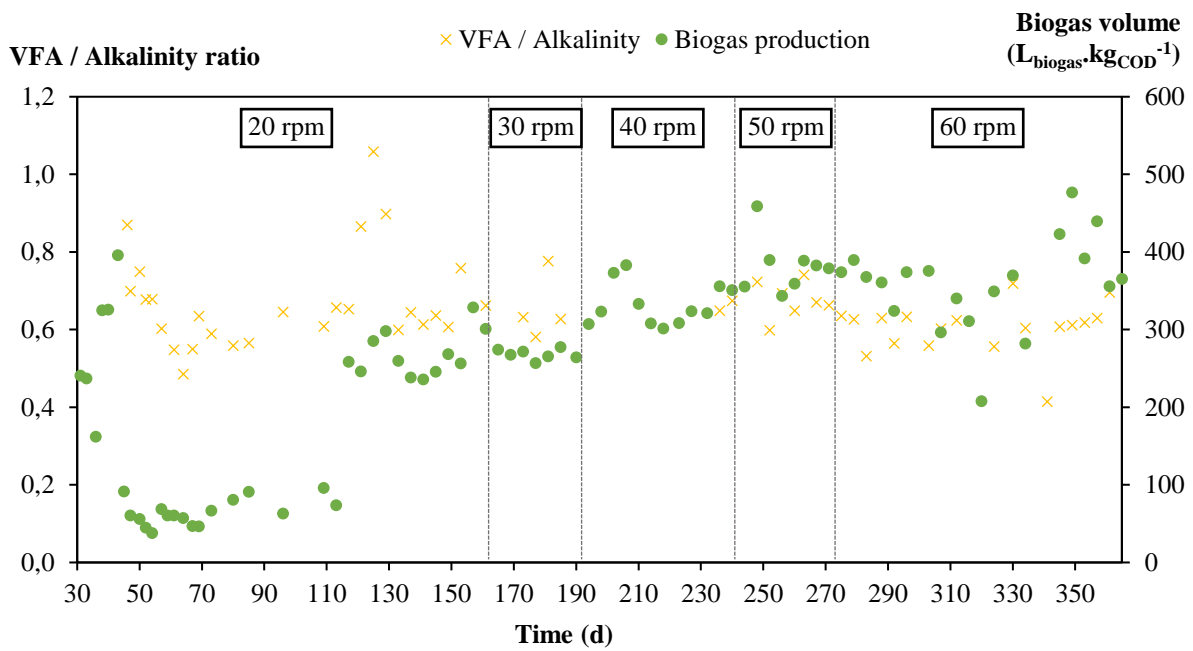
360

B



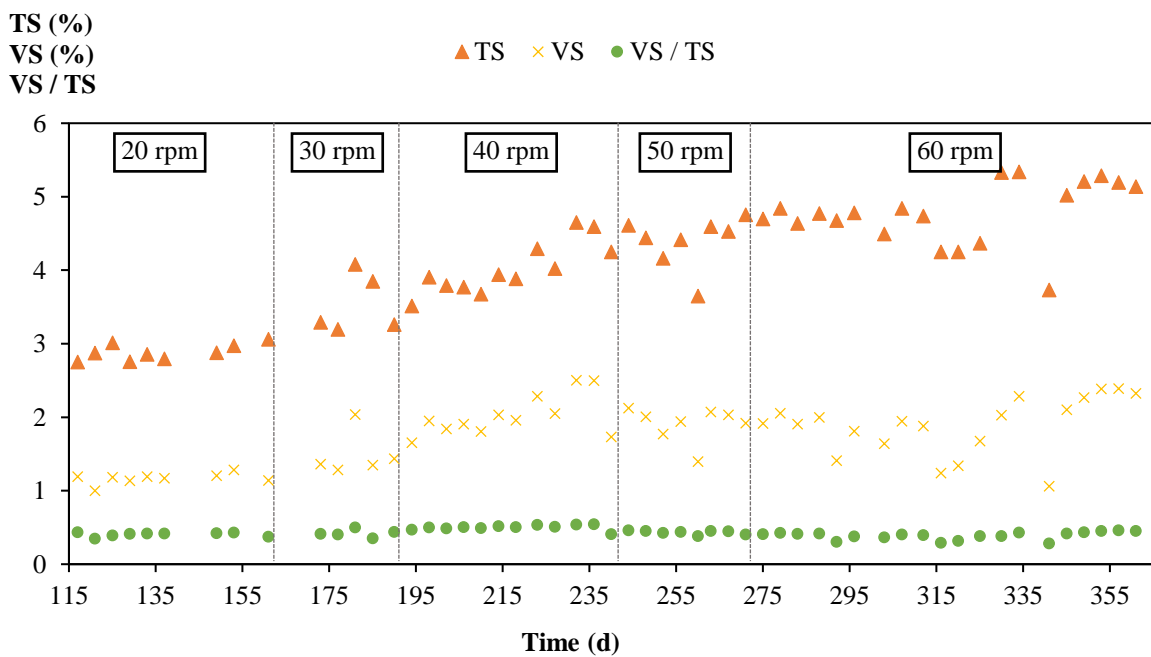
361

C



362

D



363

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

367

368 **3.4. Study of the local physicochemical properties of TOP, MID, and SLUDGE samples**

369 **3.4.1. Alkalinity and pH**

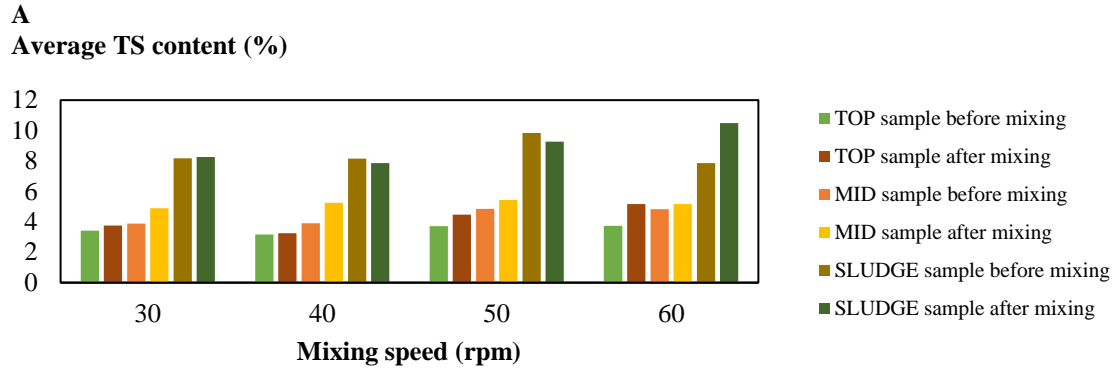
370 The alkalinity and pH before and after mixing of TOP, MID and SLUDGE samples are presented in  
371 supplementary material. The sludge had a higher alkalinity than the liquid phase. In addition, the alkalinity of  
372 TOP and MID samples was larger after than before mixing. However, the alkalinity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

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

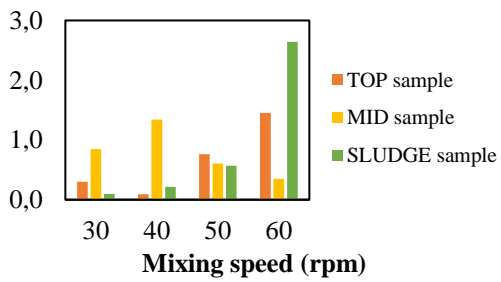
381 **3.4.2. TS and VS contents**

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

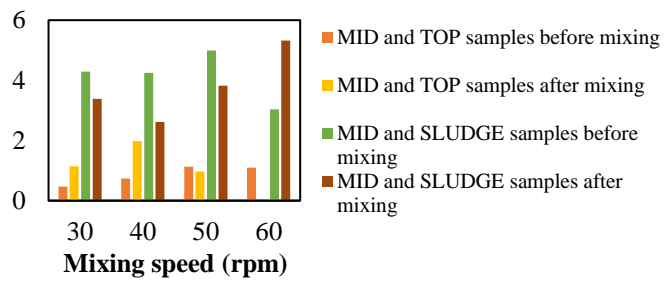
387 High TS content was observed in the SLUDGE sample. The lowest TS content was reported in TOP sample.  
388 According to the Fig. 5.C, the difference in TS content between MID and TOP samples was larger after stirring  
389 at 30 and 40 rpm, and lower after stirring at 50 and 60 rpm. Thus, stirring above 50 rpm homogenized the liquid  
390 phase of the digester. The increase in the gap is explained by the release of solid matter from the sludge to the  
391 liquid phase, resulting in a significant increase in the TS content of the MID sample as shown in Fig. 5.A and  
392 Fig. 5.B. Indeed, in Fig. 5.B we observe significant variations in TS content for the MID sample at 30, 40 and  
393 50 rpm compared to the other two samples. Similar variations of VS content occurred. The local study of TS and  
394 VS contents showed the effect of mixing on the displacement of solid matters.



**B**  
Variation of TS content before and after mixing (%)



**C**  
Difference in TS content between two samples (%)



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

398

### 399 3.4.3. VFA concentration

400 The VFA concentration before and after stirring is presented on the Fig. 6.A and the experimental data are  
401 provided in supplementary material. An increase in VFA concentration after mechanical agitation for the TOP  
402 and MID samples was observed. Indeed, variations of 0.47, 1.06, 1.81 and 4.11 g.L<sup>-1</sup> for the TOP samples were  
403 respectively obtained for the mixing speed of 30, 40, 50 and 60 rpm. The variation in VFA content was larger at  
404 60 rpm. An increase in VFA content of MID samples of 3.82, 2.98, 0.26 and 1.39 g.L<sup>-1</sup> was observed at 30, 40,  
405 50 and 60 rpm respectively. We can conclude that mixing involved an increase in the VFA concentration in the  
406 liquid phase of the digester. In addition, the greater the intensity of agitation, the greater the increase in VFA  
407 concentration is marked. These outcomes suggests that the increase in the VFA content is due to the delivery in  
408 the liquid phase of the VFAs contained in the sludge. Comparing the three samples, highest VFA content was  
409 reached in SLUDGE sample. Therefore, the VFAs have accumulated in the sludge.

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

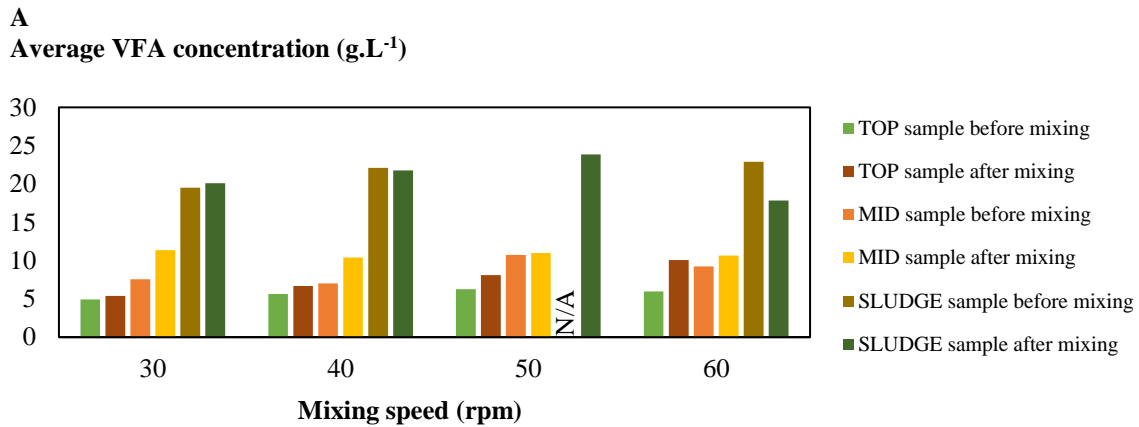
415 The biogas production decreased at 60 rpm. This reduction could be explained by the inhibitory effects of VFA.  
416 Indeed, the measurements of the digestate showed that the concentration of VFA was stable in the digester.  
417 However, the local variations in VFA content were higher at 60 rpm.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

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

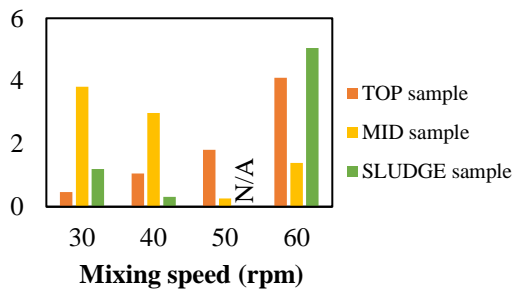


427 Considering the instabilities caused by VFA, precautions should be taken in the case of intermittent mixing as  
428 VFA accumulate in the sludge. Indeed, this work showed that the VFA accumulated in the sludge were  
429 resuspended in the liquid phase during mixing. This release of VFA into the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

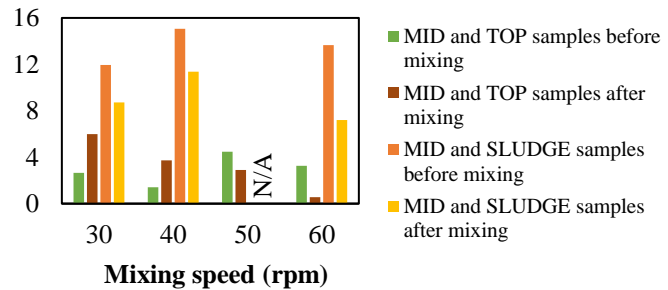
430



**B**  
Variation of VFA concentration before and after mixing (g.L<sup>-1</sup>)



**C**  
Difference in VFA concentration between two samples (g.L<sup>-1</sup>)



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

435

#### 436 4. Conclusions

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

#### 443 5. Acknowledgments

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

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562

563 **Appendices: supplementary data**

564 **Appendix A: Experimental data of the influent**

565 **Table A.1.** Feeding rate and influent concentration.

Samples and collection date	Vinasse-1	Vinasse-2	Vinasse-3	Average $\pm$ SD
	08-2018	10-2018	12-2018	
Period (d)	0-156	157-197	198-365	-
Volume added / removed (L)	1.5	1.5	1.5	1.5 $\pm$ 0
Feeding rate (L.d <sup>-1</sup> )	0.375	0.375	0.375	0.375 $\pm$ 0
OLR (g <sub>VS</sub> .L <sup>-1</sup> .d <sup>-1</sup> )	1.27	1.31	1.51	1.36 $\pm$ 0.13
OLR (g <sub>COD</sub> .L <sup>-1</sup> .d <sup>-1</sup> )	2.26	2.52	2.35	2.38 $\pm$ 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	-

566

567 **Table A.2.** Physicochemical characteristics of vinasse.

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

568

569

570 **Appendix B: Results on the biogas production**

571 **Table B.1.** Minimum, maximum, and average biogas production ( $L_{\text{biogas}} \cdot \text{kgCOD}^{-1}$ ) per mixing period.

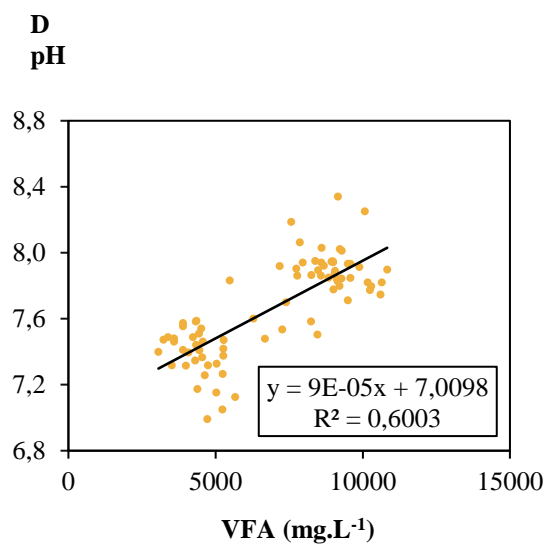
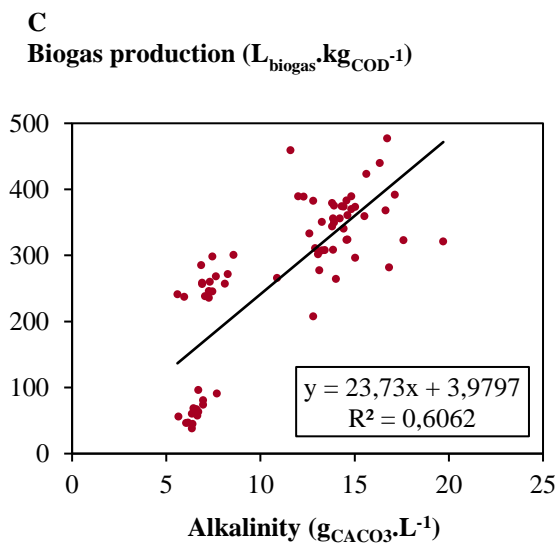
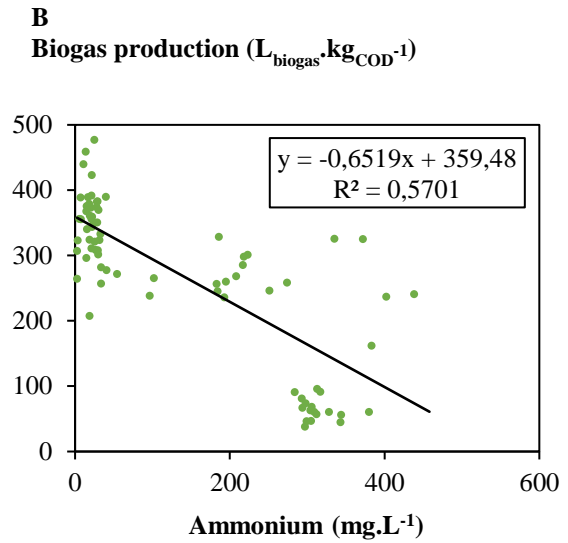
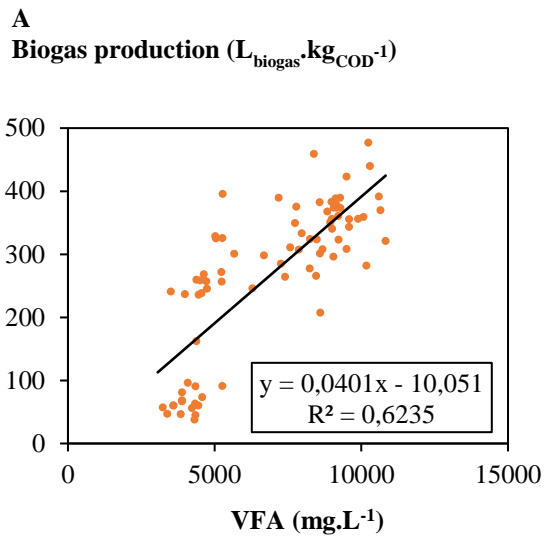
Period	Mixing speed (rpm)	Duration (days)	Minimum production	Maximum production	A1* $\pm$ SD	A2* $\pm$ SD
0-114 (Caillet and Adelard, 2020)	20	114	38	396	121 $\pm$ 106	-
115-161	20	46	236	329	268 $\pm$ 29	270 $\pm$ 32
162-190	30	28	257	278	268 $\pm$ 7	266 $\pm$ 9
191-240	40	49	302	383	332 $\pm$ 27	332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

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

573 **Table B.2.** Average methane production ( $L_{\text{CH}_4} \cdot \text{kgCOD}^{-1}$ ) in function of the proportion of methane.  
574

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	<b>215.8</b>	<b>199.2</b>	182.6	166.0	149.4
50	<b>248.3</b>	<b>229.2</b>	<b>210.1</b>	<b>191.0</b>	171.9
60	<b>233.4</b>	<b>215.4</b>	<b>197.5</b>	179.5	161.6

575



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



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

580 **Table C.1.** VFA concentration (g.L<sup>-1</sup>).

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	-

581 \*Average value, \*\*Standard deviation

582

583 **Table C.2.** Alkalinity concentration (g<sub>CaCO3</sub>.L<sup>-1</sup>).

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	-

584 \*Average value, \*\*Standard deviation

585

586

587 **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	-

588 \*Average value, \*\*Standard deviation

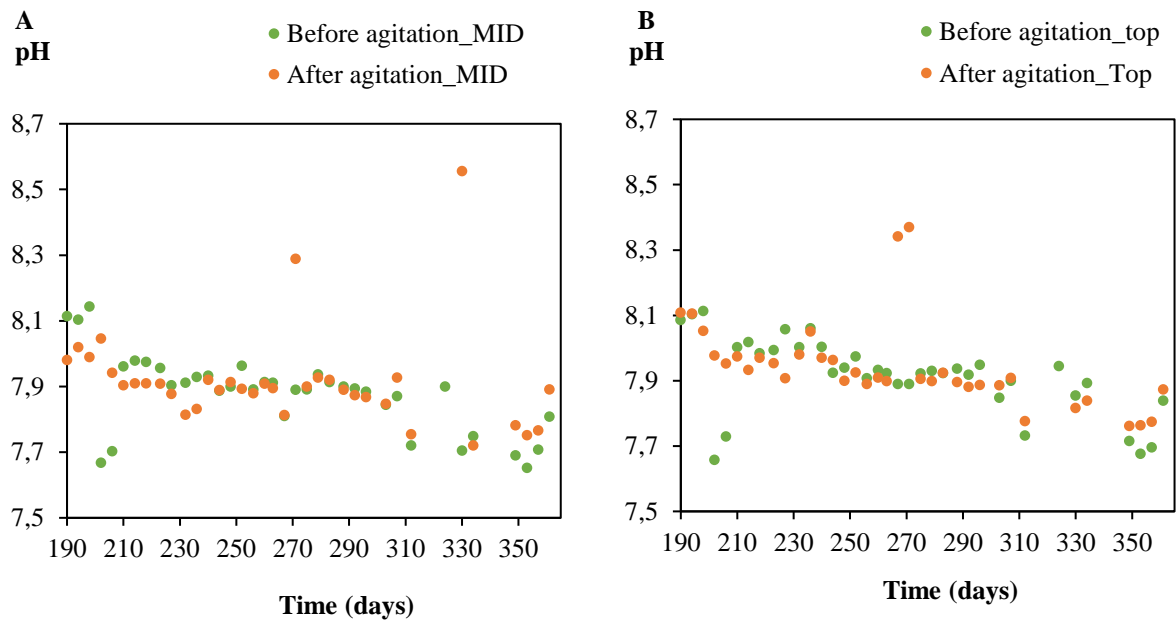
589

590 **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	-

591 \*Average value, \*\*Standard deviation

592



594 **Fig. C.1.** pH measurements before and after agitation of MID sample (A) and TOP sample (B) at 40 rpm (191-  
 595 240 days), 50 rpm (241-271 days), and 60 rpm (272-365 days).  
 596

597 **Table C.6.** Maximum, minimum and average values of physicochemical analysis of the effluent at different  
 598 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 <sup>-1</sup>	96.3	274.0	202.36	2.5	102.0	46.6	2.6	32.6	22.0
VFA	g.L <sup>-1</sup>	4.38	7.26	5.29	4.72	8.46	6.81	7.87	10.83	8.94
Alkalinity	g <sub>CaCO<sub>3</sub></sub> .L <sup>-1</sup>	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 <sup>-1</sup>	7.2	39.9	19.8	10.9	42.5	21.4	23.9	98.2	64.4
VFA	g.L <sup>-1</sup>	7.18	10.07	8.96	5.48	10.65	9.02	5.93	9.45	7.80
Alkalinity	g <sub>CaCO<sub>3</sub></sub> .L <sup>-1</sup>	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

599 \*Average value