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## Mesophilic Anaerobic Digestion of Fruit and Vegetable Waste: Single-Versus Two-Stage Reactor, and Modeling of the Liquid and Solid Fractions of the Residue

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### Abstract

The treatment of fruit and vegetable waste (FVW) is commonly carried out in continuously stirred tank reactors (CSTR). The high solids and carbohydrate content in FVW cause excessive acidification during anaerobic digestion, affecting reactor stability and methane production. The two-stage process is a possible solution to minimize this issue. To verify the CSTR as a configuration for anaerobic digestion of FVW and to propose alternatives, this study aimed to compare the single-stage with a two-stage process. The experiment evaluated hydraulic retention time of 34, 22, and 16/12 days and organic loading rates of 1.0, 1.5, and 2.0/2.5 kg chemical oxygen demand (COD)/m<sup>3</sup>·d on methane production. The two-stage system showed better performance than the single stage, resulting in a COD removal of 84% and a methane production rate of 0.459 L CH<sub>4</sub>/L·d. Furthermore, it was found that the two-stage system can lead to a reduction of 35% in the total reactor volume. In addition, the FVW digestion in a CSTR was compared with the modeling of the FVW liquid and solid fractions digestion. The modeling showed a higher potential for biomethane production with the liquid fraction (FVW<sub>L</sub>) than with raw FVW. The potential of biomethane production from the solid fraction was equal to the potential with raw FVW. Furthermore, implementing a high-rate reactor system with

FVW<sub>L</sub> could significantly reduce the total volume by 82% compared with the CSTR while also increasing methane productivity.

**Keywords:** two-stage anaerobic digestion, high-solid anaerobic digestion, food waste, biomethane, phase separation

### Introduction

The world population will reach about 9.7 billion by 2050,<sup>1</sup> leading to increased food demand. However, about 17% of the world's food is wasted, resulting in socioeconomic problems and environmental impacts.<sup>2-5</sup>

In Brazil, 10.9 million tons of fruit and vegetable waste (FVW), mostly in Wholesale Food Supply Centers (Centrais de Abastecimento S.A. - CEASA),<sup>6</sup> are produced annually, which can be used for energy production through anaerobic digestion.<sup>7</sup>

The continuous stirred tank reactor (CSTR) is commonly used to treat FVW and food waste, which has a high concentration of solids.<sup>8,9</sup> The CSTR is suitable for treating waste with high solid content because it facilitates homogenization of the material, increases interaction between the substrate and microorganisms, and reduces foam formation and temperature gradient inside the reactor. The hydraulic retention times (HRT) for CSTR range from 20 to 40 days, and a higher HRT requires a larger reactor volume, leading to higher operation costs.<sup>9-13</sup>

However, the high concentration of simple sugars in FVW can cause issues during anaerobic digestion at lower HRT and higher organic loading rate (OLR) because of the high amount of this material that enters the reactor. This is because quickly biodegradable components can cause rapid acidification. Scano et al.,<sup>14</sup> Srisowmeya et al.,<sup>15</sup> and Agrawal et al.<sup>16</sup> have identified this as a major challenge, particularly at high OLR. To prevent process failures, it is generally recommended to keep the OLR below 2–3 kg chemical oxygen demand (COD)/m<sup>3</sup>·d during the anaerobic digestion of FVW,<sup>8</sup> which results in reactors with large volumes.

One way to minimize acidification problems in FVW digestion is using a two-stage process. This process involves ensuring pH and HRT control in each stage of the anaerobic digestion process, which results in higher methane ( $\text{CH}_4$ ) production and greater stability than the single-stage process, as Agrawal et al.<sup>16</sup> stated. Another possibility unexplored to enhance methane productivity is through phase separation of the residue.<sup>17</sup> By imposing a pretreatment (grinding and centrifuging) to the FVW, a low- and a high-solid content stream, viz. a liquid and a solid phase, will be generated. The liquid phase can be subjected to low solid anaerobic digestion in a high-rate reactor. In contrast, the solid phase can be subjected to high solid anaerobic digestion in a separate high-rate reactor.

In this study, the objective was to determine the feasibility of using CSTR for FVW digestion and the effect of OLR on the process. First, the single-stage anaerobic digestion of FVW from CEASA-Maracanaú (Maracanaú, CE—Brazil) was compared with a two-stage digestion. The experiment was conducted under various HRT values (34, 22, and 16/12 days) and OLR values (1.0, 1.5, and 2.0/2.5  $\text{kg COD/m}^3\cdot\text{d}$ ). In addition, this study also aimed to propose an alternative to FVW digestion in a CSTR. The process was compared with the modeling of the separation of the liquid and solid phases of the FVW. To accomplish this, the biochemical methane potential (BMP) tests from liquid and solid fractions were compared with the unprocessed fraction.

## Material and Methods

### SUBSTRATE AND INOCULUM

The FVW used in CSTR feeding was collected at the Wholesale Food Supply Centers—CEASA in Maracanaú (CE—Brazil). The residue composition was detailed by Silva-Júnior et al.<sup>6</sup> The characterization of the FVW was performed based on analyses of COD,<sup>18</sup> total solids (TS), volatile solids (VS),<sup>18</sup> total Kjeldahl nitrogen,<sup>18</sup> total carbohydrates,<sup>19</sup> and nutrients.<sup>20</sup>

The inoculum used to start up the two methanogenic and deacidogenic reactors was a sludge from a mesophilic reactor used for brewery effluent treatment, which presented a VS concentration = 18.7 g VS/L and a specific methanogenic activity<sup>21</sup> = 0.09  $\text{m}^3 \text{CH}_4/\text{Kg VS}\cdot\text{d}$ . No adaptation was carried out to the sludge before the start-up of the methanogenic reactors. Before inoculating the acidogenic reactor, the sludge underwent heat treatment at 90°C for 10 minutes. Batches were assembled with thermally pretreated sludge, in which 1°C increased the temperature per week until reaching 43°C, the maximum accumulated hydrogen production (data not shown).

### CSTR DESIGN AND OPERATIONAL CONDITIONS

The single-stage anaerobic digestion of FVW was studied in a methanogenic CSTR (SS-CSTR) built in polyvinyl chloride, 50 cm high, 20 cm in diameter, and had a useful volume of 13 liters (Fig. 1a). The two-stage process (Fig. 1b) was evaluated with an acidogenic CSTR (TS-CSTR-1) constructed of glass, 30 cm high, 10 cm diameter (useful volume of 2 L) followed by a methanogenic CSTR (TS-CSTR-2) similar to that used in the single stage.

The feed for the reactors consisted of FVW, sodium bicarbonate (0.5 g  $\text{NaHCO}_3/\text{g COD}$ ), and water, with no nutritional

supplementation added. The HRT effect and, consequently, OLR on single-stage CSTR were evaluated by decreasing the HRT from 34 days to 22 and 16 days, thus reaching OLR 1.0, 1.5, and 2.0  $\text{kg COD/m}^3\cdot\text{d}$  (0.8, 1.3, and 1.7  $\text{kg VS/m}^3\cdot\text{d}$ ). The operational condition change was performed after at least one HRT corresponding to each stage. In the two-stage process, the acidogenic CSTR was maintained with a constant HRT of 2 days and fed at a concentration of 30  $\text{kg COD/m}^3$ , and the OLR of the reactor was 15  $\text{kg COD/m}^3\cdot\text{d}$  (12.5  $\text{kg VS/m}^3\cdot\text{d}$ ). The second-stage methanogenic CSTR was fed with the acidogenic effluent, and the HRT was 34, 20, and 12 days, applying OLR of 1.0, 1.5, and 2.5  $\text{kg COD/m}^3\cdot\text{d}$  (0.8, 1.3, and 2.0  $\text{kg VS/m}^3\cdot\text{d}$ ), respectively.

During operation, methanogenic CSTRs were kept at room temperature ( $27 \pm 4^\circ\text{C}$ ), and the acidogenic CSTR was kept in an incubator with a temperature set at 43°C. The pH of the methanogenic reactors was fixed at 7.5, and the pH of the acidogenic reactor was around 4.5.

### BATCH ASSAYS

The FVW was ground in a forage crushing and later centrifuged for 10 minutes in an industrial centrifuge to generate the liquid fraction of FVW ( $\text{FVW}_L$ ) and the solid fraction of FVW ( $\text{FVW}_S$ ). The anaerobic digestion of these three substrates (FVW,  $\text{FVW}_L$ , and  $\text{FVW}_S$ ) was compared in a mesophilic batch assay. All batch assays were carried out in 12 replicas using a flask with a reactional volume of 0.2 L filled with inoculum (5.0 g VS/L of brewery sludge), substrate (2.5 g COD/L), external buffer (2.5 g  $\text{NaHCO}_3/\text{L}$ ), and water. All flasks were purged with nitrogen gas ( $\text{N}_2$ ) for 5 minutes, sealed, and maintained under constant conditions of agitation (120 rpm) and temperature (35°C). The biogas production and composition (methane and carbon dioxide concentrations) were automatically monitored using an anaerobic respirometer (Micro-Oxymax respirometer, Columbus Instruments).

The comparative methane production and kinetic performance were achieved through five models present in Table 1. The data were fitted using no linear fit function in Origin 9.1 (Origin Lab Corporation).

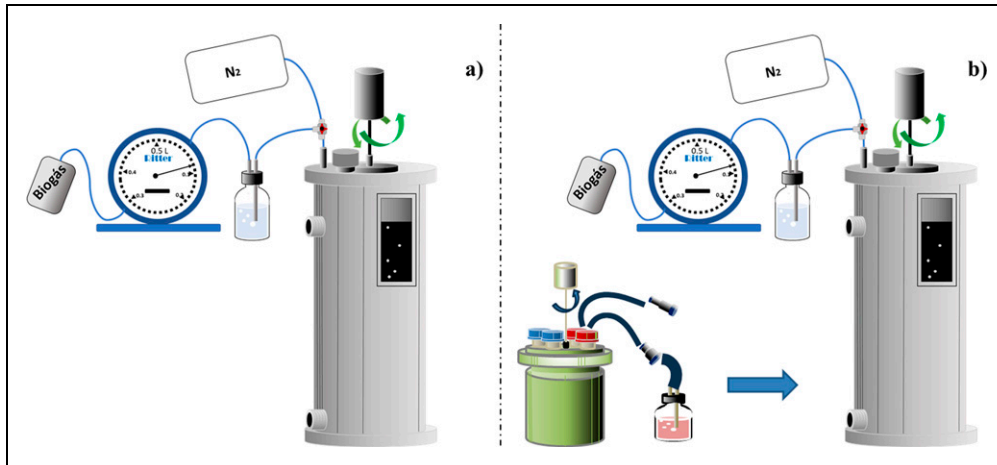
### ANALYSIS PERFORMED

The following parameters were analyzed during the operation: pH,<sup>18</sup> COD,<sup>18</sup> total carbohydrates,<sup>19</sup> total alkalinity,<sup>22</sup> volatile organic acids (VFA),<sup>23</sup> TS,<sup>18</sup> VS,<sup>18</sup> and biogas composition. High-performance liquid chromatography (Shimadzu Chromatograph with Refractive Index Detector, Model RID—M20A and Aminex HPX-87 column [Bio-Rad, 300 × 7.9 mm]) was used for the determination and quantification of acid metabolites. The biogas volume was measured in a drum-type gas meter (Ritter Apparatebau GmbH & Co. KG.), and the biogas composition determination was made by gas chromatography (C2V-200 micro-GC; Thermo-Fisher Scientific Inc.).

## Results and Discussion

### FVW CHARACTERISTICS

Table 2 presents the main parameters of FVW composition. The 13% of TS was in accordance with values between 7.4% and 17.9% observed in the literature,<sup>8</sup> which indicates that, despite the different fruits and vegetables that make up the



**Fig. 1.** Experimental apparatus used. **(a)** Single-stage anaerobic digestion containing methanogenic CSTR (SS-CSTR); **(b)** two-stage anaerobic digestion containing: (1) acidogenic CSTR (TS-CSTR-1), followed by (2) methanogenic CSTR (TS-CSTR-2). CSTR, continuously stirred tank reactors.

residue, the material presented an adequate degree of comparison with other studies that used FVW. The COD remained in 138 g COD/kg FVW, and the carbohydrate contents corresponded to 55% of the weight of FVW.

**SINGLE-STAGE CSTR PERFORMANCE**

Figure 2 show the values of methane yield (MY), methane production rate (MPR), and VFA of the SS-CSTR. The increase at the OLR promoted decreases in COD removal from 82.2% to 25.2%. Along with the decrease in organic matter degradation, higher VFA accumulations were observed as higher OLR was applied to the reactor (Fig. 2b). Such behavior may be associated with an imbalance in the microbial community as fermentative bacteria have higher growth rates than methanogenic archaea and are responsible for converting substrates to acids.<sup>24</sup> Thus, it appears that the adoption of smaller HRT overloaded the reactor and was not adequate for the activity of methanogenic microorganisms. In addition, the VFA accumulation and instabilities in the anaerobic digestion of FVW can be related to the composition of the waste, which has a high proportion of carbohydrates (55%) (Table 2), materials known for their easy assimilation and conversion by bacteria.

The accumulation of VFA because of increases at the OLR in single-stage anaerobic digestion of FVW was also observed in the literature.<sup>7,12</sup>

The instability in the SS-CSTR generated at the OLR of 2.0 kg COD/m<sup>3</sup>·d can also be identified by the high ratio between intermediate alkalinity and partial alkalinity (IA/PA ratio), =1.2 (16 hours HRT). At OLR 1.0 and 1.5 kg COD/m<sup>3</sup>·d, the IA/PA ratio was 0.4 and 0.3, in agreement with the stability value of 0.3 indicated in the literature.<sup>22</sup>

MY and MPR showed a decrease as OLR increased (Fig. 2), with the OLR being 1.0 kg COD/m<sup>3</sup>·d, in which HRT was 34 days, the operational condition with higher MY and MPR. The increase at the OLR to 2.0 kg COD/m<sup>3</sup>·d caused a decrease of 68% and 46% in MY and MPR, respectively. In an analysis of the variance test, it was discovered that there are statistically significant differences between the means of MY and MPR (*p* value <0.05). Further analysis using the Tukey’s test revealed that except for the MPR of OLR 1.0 and 1.5 kg COD/m<sup>3</sup>·d, all other MPR differed significantly from one another. As occurred with MY and MPR, the methane composition in the biogas also decreased as higher OLR were used, falling from 61% at 1.0 kg COD/m<sup>3</sup>·d to 36% at 2.0 kg COD/m<sup>3</sup>·d. It can be seen in

**Table 1. Kinetic Models Used to Describe Biogas Production**

|  |                                   |
|--|-----------------------------------|
| $M_{(t)} = M_0 \cdot \exp\left\{-\exp\left[\frac{R_m \cdot t}{M_0} \cdot (\lambda - t) + 1\right]\right\}$ | Gompertz–GoM (Eq. 1)              |
| $M_{(t)} = \frac{M_0}{1 + \exp(4 \cdot R_m \cdot (\lambda - t) / M_0 + 2)}$                                | Logistic–LM (Eq. 2)               |
| $M_{(t)} = M_0 \cdot (1 - \exp(-R_m \cdot (t - \lambda) / M_0))$   | Transference function–TFM (Eq. 3) |
| $M_{(t)} = M_0 \cdot \{1 - e^{-k_h \cdot t}\}$   | Degradation–DM (Eq. 4)            |
| $M_{(t)} = M_0 \times \{1 - (\gamma / \gamma + t)^r\}$   | Gamma–GaM (Eq. 5)                 |

$\lambda$ , lag phase (d);  $k_h$ , first-order degradation coefficient (d);  $M_t$ , cumulative methane production (NmL CH<sub>4</sub>/g COD);  $M_0$ , maximal biomethane production potential (NmL CH<sub>4</sub>/g COD);  $r, \gamma$ , constant coefficient (d);  $R_m$ , maximal methane production rate (NmL CH<sub>4</sub>/g COD·d);  $t$ , time (d).

**Table 2. Characterization of FVW,<sup>6</sup> FVW<sub>S</sub>, and FVW<sub>L</sub>**

|                                   | FVW  | FVW <sub>S</sub> | FVW <sub>L</sub> |
|-----------------------------------|------|------------------|------------------|
| COD (g/kg ww)                     | 138  | 201              | 116              |
| Carbohydrates (% ww)              | 55   | 63               | 46               |
| Lipids (% ww)                     | 0.8  | 1.6              | 0.8              |
| Crud proteins (% ww)              | 2.3  | 1.3              | 1.2              |
| Total solids (%)                  | 13   | 28               | 11               |
| Volatile solids (%)               | 12.2 | 27.5             | 10.3             |
| Total Kjeldahl nitrogen (g/kg dw) | 9.3  | NA               | NA               |

COD, chemical oxygen demand; dw, dry weight; FVW, fruit and vegetable waste; FVW<sub>L</sub>, liquid fraction of FVW; FVW<sub>S</sub>, solid fraction of FVW; NA, not applicable; ww, wet weight.

Fig. 2b that the decrease in MY was accompanied by an increase in the concentration of VFA in the SS-CSTR and that the OLR 2.0 kg COD/m<sup>3</sup>·d, whose lowest MY and MPR were observed, was the one that presented the highest VFA accumulation (2610 mg HAc/L). According to Chew et al.,<sup>25</sup> VFA concentration above 4 g/L could decrease significantly methane production. However, according to De Vrieze et al.,<sup>26</sup> the inhibitory concentration is above 3 g/L. The exact inhibitory value will depend on substrate composition and operational conditions.<sup>27</sup> In the SS-CSTR, the high concentrations of VFA may have affected the methanogenic archaea, microorganisms responsible for methane production.

Table 3 presents studies that applied CSTR for single-stage anaerobic digestion of FVW. It is verified that HRT has a direct effect on methane production as higher MY in the mono-digestion of FVW in a single-stage process occurred in the highest HRT of 40 and 80 days (480 and 450 mL CH<sub>4</sub>/g VS), with OLR of 2.5 and 2.0 kg VS/m<sup>3</sup>·d, respectively.<sup>28,29</sup>

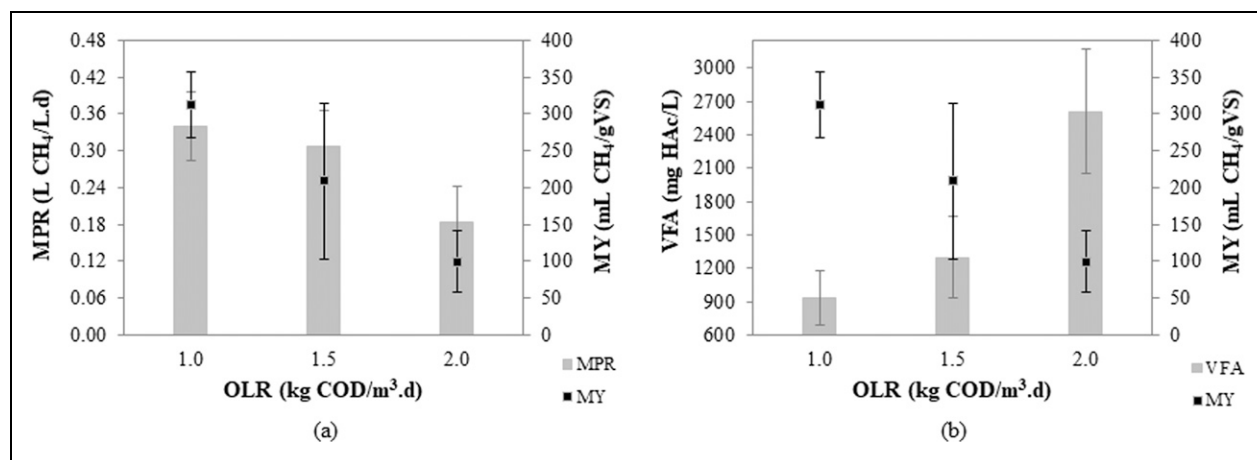
Because of the results presented in this study, it is observed that the performance of the single-stage mesophilic CSTR for

mono-digestion of FVW was similar to the results commonly described in the literature, presenting its maximum performance at low OLR (1.0 kg COD/m<sup>3</sup>·d or 0.8 kg VS/m<sup>3</sup>·d). It has been noted that even with a low OLR, the single-stage CSTR may not be the ideal choice for FVW treatment because of its subpar performance and instability issues when OLR is increased. An alternative option is to consider a two-stage anaerobic digestion (TSAD) process, where the fermentation phase is separated from the methanogenic phase with conditions tailored to each stage of anaerobic digestion.

## TWO-STAGE CSTR PERFORMANCE

The TSAD process consisted of using an acidogenic CSTR in the first stage (TS-CSTR-1) whose acidified effluent fed a methanogenic CSTR in the second stage (TS-CSTR-2). The consumption of carbohydrates in TS-CSTR-1 was 50 ± 16%, and the pH was 4.3 ± 0.2. Thus, lactic acid (HLA), acetic acid (HAc), and isobutyric acid (HIsBu) were identified at concentrations of 8082 ± 466 mg HLA/L, 2586 ± 130 mg HAc/L, and 342 ± 46 mg HIsBu/L, respectively. In FVW fermentation, HLA production is commonly observed to be favored at pH 4.0–5.0.<sup>30–33</sup> The production of HLA in the acidogenic reactor of the TSAD process can be beneficial for methane production as the conversion of lactate to acetate is more spontaneous than that of butyrate and propionate to acetate.<sup>31</sup>

TS-CSTR-1 effluent was directed to feed TS-CSTR-2 after the acidogenic step. The COD removal rate was 84%, the VFA concentration was 793 mg HAc/L, and the IA/PA ratio was 0.3 at OLR 1.5 kg COD/m<sup>3</sup>·d. TS-CSTR-2 showed greater robustness and higher stability than the single-stage process at OLR 1.5 kg COD/m<sup>3</sup>·d. The TSAD process allowed for a 50% increase in the CSTR's OLR without compromising its operation. Shen et al.<sup>34</sup> observed that a TSAD process allowed for greater treatment capacity in a mesophilic CSTR, allowing for an OLR of 33.3% higher than that of the single-stage process. This could be attributed to optimized parameters for each stage of anaerobic digestion. However, COD removal decreased to 26%, and VFA accumulation was high at 1744 mg HAc/L,



**Fig. 2.** Production of methane and volatile acids in the single-stage methanogenic reactor as a function of the applied OLR. (a) MPR and MY; (b) VFA and MY. MPR, methane production rate; MY, methane yield; OLR, organic loading rates; VFA, volatile organic acids.

**Table 3. Comparison of the Main Results of the FVW Treatment in CSTR Using Single-Stage Anaerobic Digestion**

| INOCULUM             | TEMPERATURE | OLR                         | HRT     | REMOVAL OF ORGANIC MATTER | MY                           | MPR                            | REFERENCE                    |
|----------------------|-------------|-----------------------------|---------|---------------------------|------------------------------|--------------------------------|------------------------------|
| Granular sludge      | 35°C        | 2 kg VS/m <sup>3</sup> ·d   | 80 days | 82% (VS)                  | 450 mL CH <sub>4</sub> /g VS | –                              | Ganesh et al. <sup>29</sup>  |
| Mesophilic digestate | 35°C        | 4.8 kg VS/m <sup>3</sup> ·d | 20 days | 82% (VS)                  | 403 mL CH <sub>4</sub> /g VS | 1.8–2.2 L CH <sub>4</sub> /L·d | Arhoun et al. <sup>30</sup>  |
| Mesophilic digestate | 37°C        | 3 kg VS/m <sup>3</sup> ·d   | 30 days | –                         | 285 mL CH <sub>4</sub> /g VS | 0.86 L CH <sub>4</sub> /L·d    | Edwiges et al. <sup>12</sup> |
| Anaerobic sludge     | 35°C        | 2.5 kg VS/m <sup>3</sup> ·d | 40 days | 83.1% (COD)<br>82.7% (VS) | 480 mL CH <sub>4</sub> /g VS | –                              | Jo et al. <sup>28</sup>      |
| Anaerobic sludge     | 30°C        | 0.8 kg VS/m <sup>3</sup> ·d | 34 days | 82.2% (COD)               | 312 mL CH <sub>4</sub> /g VS | 0.339 L CH <sub>4</sub> /L·d   | This study                   |

CSTR, continuously stirred tank reactors; HRT, hydraulic retention time; MPR, methane production rate; MY, methane yield; OLR, organic loading rate.

resulting in a period of instability with an IA/PA ratio of 1.4 when OLR was increased to 2.5 kg COD/m<sup>3</sup>·d (HRT 12 days).

The same behavior identified in the single-stage process was observed in the TSAD process. The increase at the OLR through decreases at the HRT resulted in an overload of the reactor, generating higher accumulations of VFA and instabilities and, consequently, lower MY and MPR. Viturtia et al.<sup>35</sup> observed a drop in MY from 0.4 to 0.1 L CH<sub>4</sub>/g VS when increasing the OLR from 3.1 to 12.6 kg VS/m<sup>3</sup>·d because of the accumulation of VFA in the reactor. Bouallagui et al.<sup>33</sup> verified an increase from 342 to 432 mL CH<sub>4</sub>/g VS when increasing the OLR from 0.5 to 1.2 kg VS/m<sup>3</sup>·d. However, the aforementioned authors applied HRT of 10 days in the methanogenic reactor of the TSAD process and used lower OLR than those adopted in the TS-CSTR-2. When comparing the MY of 432 mL CH<sub>4</sub>/g VS and the MPR of 0.5 L CH<sub>4</sub>/L·d determined by Bouallagui et al.<sup>33</sup> at the OLR of 1.2 kg VS/m<sup>3</sup>·d with MY of 428 mL CH<sub>4</sub>/g VS and MPR of 0.46 L CH<sub>4</sub>/L·d of TS-CSTR-2 at the OLR of 1.3 kg VS/m<sup>3</sup>·d (1.5 kg COD/m<sup>3</sup>·d), similar values are observed, regardless of the HRT and the reactor type.

Van et al.<sup>36</sup> investigated the TSAD of vegetable waste using an upflow anaerobic sludge blank reactor and HRT of 20 days in the methanogenic stage and observed greater MY when increasing the OLR from 0.33 to 1.23 kg COD/m<sup>3</sup>·d, reaching 303.4 mL CH<sub>4</sub>/g VS<sub>add</sub>. In contrast to the OLR commonly used for two-stage digestion of FVW, Dinh et al.<sup>37</sup> applied the OLR of 7.6 kg VS/m<sup>3</sup>·d and HRT of 5 days in an upflow reactor with an anaerobic organism layer at the bottom and verified MY of 306 mL CH<sub>4</sub>/g VS. Despite the high OLR compared with this study, Dinh et al.<sup>37</sup> obtained MY below that found in TS-CSTR-2. So, for mesophilic treatment of FVW in two stages, the OLR is the key parameter for obtaining better biogas production. According to the authors and their study, it has been observed that the highest value of MY for FVW digestion is nearly 0.4 L CH<sub>4</sub>/g VS.

Table 4 presents the main results of the methanogenic reactors evaluated in this study, that is, the SS-CSTR and TS-CSTR-2. According to the study, using a two-stage process enables smaller HRTs than a single-stage process. As a result, the total reactor volume decreased by 35%, reducing investment costs. TS-CSTR-2 also achieved higher MY and MPR values (37% and 35%, respectively) than SS-CSTR. In addition, TS-CSTR-2 produced 196 kJ/d

in energy production, whereas SS-CSTR produced 145 kJ/d, assuming a calorific value of 50 MJ/kg for methane. However, the economic feasibility of using TS-CSTR-1 followed by TS-CSTR-2 must be further evaluated in the future, as it requires constructing and operating two reactors.

Conducting a mass balance for the two methanogenic reactors, the maximum possible MPR for the SS-CSTR reactor, applying an OLR of 1.0 g COD/L·d, would be 0.35 L CH<sub>4</sub>/L·d. However, with COD removed equal to 82.2%, this value decreases to 0.29 L CH<sub>4</sub>/L·d. Therefore, the experimental MPR of 0.34 L CH<sub>4</sub>/L·d is higher than the theoretical one. This difference may be attributed to COD removal analyses, which might have accounted for COD from the sludge and/or experienced other interferences in its measurement. In the TS-CSTR, the theoretical MPR for an OLR of 1.5 g COD/L·d and 84% COD removal is 0.44 L CH<sub>4</sub>/L·d. Consequently, an experimental value of 0.46 L CH<sub>4</sub>/L·d, similar to the theoretical one, is observed, indicating the good performance of the process.

**BATCH ASSAYS WITH THE LIQUID AND SOLID FRACTIONS (FVW, FVW<sub>L</sub>, AND FVW<sub>S</sub>)**

The high sludge activity and the high volatile acid content in all substrates supported the rapid methanogenesis process, which was verified through a short lag phase. For this reason,

**Table 4. Main Results of the Methanogenic Reactors of Single-Stage (SS-CSTR) and Two-Stage (TS-CSTR-2) Anaerobic Digestion of FVW**

|                                | SS-CSTR | TS-CSTR-2 |
|--------------------------------|---------|-----------|
| OLR (kg COD/m <sup>3</sup> ·d) | 1.0     | 1.5       |
| HRT (d)                        | 34      | 20        |
| COD removed (%)                | 82.2    | 84 %      |
| MY (mL CH <sub>4</sub> /g·VS)  | 312     | 428       |
| MPR (L CH <sub>4</sub> /L·d)   | 0.34    | 0.46      |
| Energy production (kJ/d)       | 158     | 214       |

**Table 5. Summary of Fitted Results of Biogas and Methane Yields Parameters According to Evaluated Models (Gompertz, Logistic, Transference Function, Degradation, and Gamma)**

| BASE             | PARAMETER                                    | FVW   | FVW <sub>L</sub> | FVW <sub>S</sub> |
|------------------|--|-------|------------------|------------------|
| Characterization | TS (%)                                       | 13    | 7.9              | 18.3             |
|                  | VS (%)                                       | 12    | 7.2              | 16.8             |
|                  | COD (g/L)                                    | 0.14  | 0.11             | 0.18             |
| GoM              | M <sub>0</sub> (mL CH <sub>4</sub> /g COD)   | 297   | 312              | 297              |
|                  | R <sub>m</sub> (mL CH <sub>4</sub> /g COD-d) | 104   | 117              | 69               |
|                  | R <sup>2</sup>                               | 0.884 | 0.862            | 0.912            |
| LM               | M <sub>0</sub> (mL CH <sub>4</sub> /g COD-d) | 297   | 311              | 296              |
|                  | R <sub>m</sub> (mL CH <sub>4</sub> /g COD-d) | 86    | 99               | 56               |
|                  | R <sup>2</sup>                               | 0.863 | 0.838            | 0.891            |
| TFM              | M <sub>0</sub> (mL CH <sub>4</sub> /g COD-d) | 297   | 312              | 297              |
|                  | R <sub>m</sub> (mL CH <sub>4</sub> /g COD-d) | 224   | 247              | 153              |
|                  | R <sup>2</sup>                               | 0.912 | 0.891            | 0.937            |
| DM               | M <sub>0</sub> (mL CH <sub>4</sub> /g COD-d) | 296   | 311              | 295              |
|                  | k <sub>n</sub> (d)                           | 1.0   | 1.0              | 0.71             |
|                  | R <sup>2</sup>                               | 0.896 | 0.878            | 0.915            |
| GaM              | M <sub>0</sub> (mL CH <sub>4</sub> /g COD-d) | 296   | 311              | 295              |
|                  | R <sup>2</sup>                               | 0.895 | 0.877            | 0.915            |

TS, total solids; VS, volatile solids.

the lag phase for all substrate assays was removed for the fitted parameters summarized in *Table 5*.

The five models returned statistically equal M<sub>0</sub> values but different R<sub>m</sub> for all substrates, with FVW<sub>L</sub> as the highest value and FVW<sub>S</sub> as the lowest. This difference stems from the lower particulate content of FVW<sub>L</sub> compared with FVW<sub>S</sub>. According to Vavilin et al.,<sup>38</sup> the high particulate content limits organic material digestion and hydrolysis rates. In this line, Palmowski and Müller<sup>39</sup> evaluated the anaerobic digestion of FW under different particulate contents, observing more significant limitations of the hydrolysis rate for high particulate content. Intriguingly, the k<sub>n</sub> of the FVW was the same as the FVW<sub>L</sub> but 30% less for the FVW<sub>S</sub>. Commonly, k<sub>n</sub> values greater than 0.5 indicate high biodegradability to FVW degradation.<sup>38,40</sup> On the contrary, kinetic fit models that follow the first order may not faithfully reflect the degradation process, especially for complex substrates.<sup>41</sup> Silva et al.<sup>41</sup> recently proposed the Gama as the most suitable model for more complete substrates. Positively, the M<sub>0</sub> found through the Gama model was equal to the value adjusted in the other models (*p* value >0.05).

#### CSTR TECHNOLOGY FOR THE ANAEROBIC DIGESTION OF FVW

Although the literature indicates the CSTR as the best option for waste treatment with high total solids content,<sup>15</sup> it is verified in this study that the maximum OLR possible to be used without

generating process instability is low. As FVW is generated in large quantities and is mainly composed of organic matter, the most interesting treatment would be the FVW at a higher OLR. Using co-substrates with FVW could favor the performance of the methanogenic step and, thus, collaborate to insert larger OLR, requiring further studies on the subject. Furthermore, adopting thermophilic conditions could favor the adoption of higher OLR.<sup>37</sup>

Besides, the batch tests with the fractions separated in FVW<sub>L</sub> and FVW<sub>S</sub> demonstrated that the anaerobic digestion of liquid and solid phases is potentially exploitable. In the case of FVW<sub>L</sub>, there is the possibility of applying high-rate reactors, which can reach higher OLR than that found in CSTR studied here (OLR > 10 kg COD/m<sup>3</sup>·d) with methane production up to 2 m<sup>3</sup> CH<sub>4</sub>/m<sup>3</sup>·d.<sup>42</sup> In the case of FVW<sub>S</sub>, there is the possibility of using dry digestion reactors, which support high solid content (>20% TS) with OLR also >10 kg VS/m<sup>3</sup>·d and methane production up to 14.1 m<sup>3</sup> CH<sub>4</sub>/m<sup>3</sup>·d.<sup>43</sup> In this case, considering the concentration of organic matter in each waste (*Table 2*), the volume of waste to be treated by each system (in the case of FVW<sub>L</sub> and FVW<sub>S</sub>), and the loading rates of each system, it is concluded that the high-rate system is capable of treating the same volume of waste generated, 17 tons per day in the case of CEASA,<sup>6</sup> in a significantly smaller reactor volume. Thus, implementing a high-rate reactor system could significantly reduce the total volume by 82% compared with the CSTR while also increasing methane productivity.

## Conclusions

The two-stage process showed better performance than the single-stage process in the FVW treatment under similar conditions. With the occurrence of the acidogenesis and methanogenesis stages in different reactors, it was possible to increase the OLR of the methanogenic reactor by 50% and obtain energy production 35% higher, corresponding to 196 kJ/d. However, the maximum applied OLR was 1.5 kg COD/m<sup>3</sup>·d. Thus, the CSTR may not be the most suitable type of reactor for FVW mono-digestion, as large amounts of this waste are generated and, therefore, higher OLR are required for its treatment. The choice to separate the liquid and solid fractions proved to be a wiser decision. The liquid fraction had a higher potential for biomethane production compared with raw FVW, which was equal to that of the solid fraction. In addition, implementing a high-rate reactor system could significantly reduce the total volume by 82% compared with the CSTR while also increasing methane productivity. It's important to note that this could lead to greater efficiency and cost savings.

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## Authors' Contributions

The authors confirm contribution to the article as follows: Study conception and design: C.A.D.M. and R.C.L. Data collection: N.F.P. and F.D.C.G.D.S.J. Interpretation of results: P.D.S.A. and W.D.A.C. Draft article preparation: P.D.S.A., W.D.A.C., and C.A.D.M. All authors reviewed the results and approved the final version of the article.

## Author Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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## REFERENCES

- United Nations. World Population Prospects 2022: Summary of Results. New York; 2022.
- FAO - Food and Agriculture Organization of the United Nations. World Food and Agriculture—Statistical Yearbook 2022. FAO: Rome; 2022; doi: 10.4060/cc2211en
- UNEP - United Nations Environment Programme. Food Waste Index Report 2021. Nairobi; 2021.
- Scherhauser S, Moates G, Hartikainen H, et al. Environmental impacts of food waste in Europe. *Waste Manag* 2018;77:98–113; doi: 10.1016/j.wasman.2018.04.038
- O'Connor J, Mickan BS, Rinklebe J, et al. Environmental implications, potential value, and future of food-waste anaerobic digestate management: A review. *J Environ Manage* 2022;318:115519; doi: 10.1016/j.jenvman.2022.115519
- Silva Júnior FdCGd, Menezes CA, de Cavalcante WA, et al. Characterization of fruits and vegetables waste generated at a central horticultural wholesaler: A case study for energy production via biogas. *Industrial Biotechnology* 2022; 18(4):235–239; doi: 10.1089/ind.2021.0032
- Cavalcante WA, de Menezes CA, da Silva Júnior FCG, et al. From start-up to maximum loading: An approach for methane production in upflow anaerobic sludge blanket reactor fed with the liquid fraction of fruit and vegetable waste. *J Environ Manage* 2023;335:117578; doi: 10.1016/j.jenvman.2023.117578
- Xu F, Li Y, Ge X, et al. Anaerobic digestion of food waste—Challenges and opportunities. *Bioresour Technol* 2018;247:1047–1058; doi: 10.1016/j.biortech.2017.09.020
- Megido L, Negral L, Fernández-Nava Y, et al. Impact of organic loading rate and reactor design on thermophilic anaerobic digestion of mixed supermarket waste. *Waste Manag* 2021;123:52–59; doi: 10.1016/j.wasman.2021.01.012
- Fagbohunge MO, Dodd IC, Herbert BMJ, et al. High solid anaerobic digestion: Operational challenges and possibilities. *Environ Technol Innov* 2015;4:268–284; doi: 10.1016/j.eti.2015.09.003
- Wang F, Zhang C, Huo S. Influence of fluid dynamics on anaerobic digestion of food waste for biogas production. *Environ Technol* 2017;38(9):1160–1168; doi: 10.1080/09593330.2016.1220429
- Edwige T, Frare LM, Lima Alino JH, et al. Methane potential of fruit and vegetable waste: An evaluation of the semi-continuous anaerobic mono-digestion. *Environ Technol* 2020;41(7):921–930; doi: 10.1080/09593330.2018.1515262
- Assis TI, Gonçalves RF. Valorization of food waste by anaerobic digestion: A bibliometric and systematic review focusing on optimization. *J Environ Manage* 2022;320:115763; doi: 10.1016/j.jenvman.2022.115763
- Scano EA, Asquer C, Pistis A, et al. Biogas from anaerobic digestion of fruit and vegetable wastes: Experimental results on pilot-scale and preliminary performance evaluation of a full-scale power plant. *Energy Convers Manag* 2014;77: 22–30; doi: 10.1016/j.enconman.2013.09.004
- Srisowmeya G, Chakravarthy M, Nandhini Devi G. Critical considerations in two-stage anaerobic digestion of food waste—A review. *Renewable and Sustainable Energy Reviews* 2020;119:109587; doi: 10.1016/j.rser.2019.109587
- Agrawal A, Chaudhari PK, Ghosh P. Anaerobic digestion of fruit and vegetable waste: A critical review of associated challenges. *Environ Sci Pollut Res Int* 2022;30(10):24987–25012; doi: 10.1007/s11356-022-21643-7
- Chen L, He Z, Yang L, et al. Optimal utilization of solid residue from phase-separation pretreatment before food waste anaerobic digestion. *J Clean Prod* 2022;372:133795; doi: 10.1016/j.jclepro.2022.133795
- APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater. 22nd ed. (Rice EW, Baird RB, Eaton AD. Eds). American Public Health Association: Washington, DC, USA; 2012.
- DuBois M, Gilles KA, Hamilton JK, et al. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956;28(3):350–356; doi: 10.1021/ac60111a017
- Miyazawa M, Pavan MA, Muraoka T, et al. Análise Química de Tecido Vegetal. In: Manual de Análises Químicas de Solos, Plantas e Fertilizantes Embrapa Informação Tecnológica; Embrapa Soils: Rio de Janeiro; 2009.
- Soto M, Méndez R, Lema JM. Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental set up. *Water Res* 1993;27(8): 1361–1376; doi: 10.1016/0043-1354(93)90224-6
- Ripley LE, Boyle WC, Converse JC. Improved alkalimetric monitoring for anaerobic Digestion of high-strength wastes. *J Water Pollut Control Fed* 1986;58(5): 406–411.
- DiLallo R, Albertson OE. Volatile Acids by Direct Titration. *J Water Pollut Control Fed* 1961;33(4):356–365.

24. Harirchi S, Wainaina S, Sar T, et al. Microbiological insights into anaerobic digestion for biogas, hydrogen or volatile fatty acids (VFAs): A review. *Bioengineered* 2022;13(3):6521–6557; doi: 10.1080/21655979.2022.2035986
25. Chew KR, Leong HY, Khoo KS, et al. Effects of anaerobic digestion of food waste on biogas production and environmental impacts: A review. *Environ Chem Lett* 2021;19(4):2921–2939; doi: 10.1007/s10311-021-01220-z
26. de Vrieze J, Hennebel T, Boon N, et al. Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresour Technol* 2012;112:1–9; doi: 10.1016/j.biortech.2012.02.079
27. Ahring BK, Sandberg M, Angelidaki I. Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Appl Microbiol Biotechnol* 1995; 43(3):559–565; doi: 10.1007/BF00218466
28. Jo Y, Rhee C, Choi H, et al. Long-term effectiveness of bioaugmentation with rumen culture in continuous anaerobic digestion of food and vegetable wastes under feed composition fluctuations. *Bioresour Technol* 2021;338:125500; doi: 10.1016/j.biortech.2021.125500
29. Ganesh R, Torrijos M, Sousbie P, et al. Single-phase and two-phase anaerobic digestion of fruit and vegetable waste: Comparison of start-up, reactor stability and process performance. *Waste Manag* 2014;34(5):875–885; doi: 10.1016/j.wasman.2014.02.023
30. Arhoun B, Villen-Guzman MD, Vereda-Alonso C, et al. Anaerobic co-digestion of municipal sewage sludge and fruit/vegetable waste: Effect of different mixtures on digester stability and methane yield. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2019;54(7):628–634; doi: 10.1080/10934529.2019.1579523
31. Wu Y, Wang C, Liu X, et al. A new method of two-phase anaerobic digestion for fruit and vegetable waste treatment. *Bioresour Technol* 2016;211:16–23; doi: 10.1016/j.biortech.2016.03.050
32. Wu Y, Ma H, Zheng M, et al. Lactic acid production from acidogenic fermentation of fruit and vegetable wastes. *Bioresour Technol* 2015;191:53–58; doi: 10.1016/j.biortech.2015.04.100
33. Bouallagui H, Torrijos M, Godon JJ, et al. Two-phases anaerobic digestion of fruit and vegetable wastes: Bioreactors performance. *Biochem Eng J* 2004; 21(2):193–197; doi: 10.1016/j.bej.2004.05.001
34. Shen F, Yuan H, Pang Y, et al. Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): Single-phase vs. two-phase. *Bioresour Technol* 2013;144:80–85; doi: 10.1016/j.biortech.2013.06.099
35. Mtz.-Vituria A, Mata-Alvarez J, Cecchi F. Two-phase continuous anaerobic digestion of fruit and vegetable wastes. *Resour Conserv Recycl* 1995;13(3–4): 257–267; doi: 10.1016/0921-3449(94)00048-A
36. Pham Van D, Takeshi F, Hoang Minh G, et al. Comparison between single and two-stage anaerobic digestion of vegetable waste: Kinetics of methanogenesis and carbon flow. *Waste Biomass Valor* 2020;11(11):6095–6103; doi: 10.1007/s12649-019-00861-0
37. Dinh PV, Fujiwara T. Biogas production and energy balance in a two-stage anaerobic digestion of fruit and vegetable waste: Thermophilic versus mesophilic. *Fermentation* 2023;9(7):601; doi: 10.3390/fermentation9070601
38. Vavilin VA, Fernandez B, Palatsi J, et al. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Manag* 2008; 28(6):939–951; doi: 10.1016/j.wasman.2007.03.028
39. Palmowski LM, Müller JA. Influence of the size reduction of organic waste on their anaerobic digestion. *Water Sci Technol* 2000;41(3):155–162; doi: 10.2166/wst.2000.0067
40. Browne JD, Allen E, Murphy JD. Improving hydrolysis of food waste in a leach bed reactor. *Waste Manag* 2013;33(11):2470–2477; doi: 10.1016/j.wasman.2013.06.025
41. Da Silva C, Peces M, Faundez M, et al. Gamma distribution function to understand anaerobic digestion kinetics: Kinetic constants are not constant. *Chemosphere* 2022;306:135579; doi: 10.1016/j.chemosphere.2022.135579
42. Mainardis M, Buttazzoni M, Goi D. Up-Flow anaerobic sludge blanket (UASB) technology for energy recovery: A review on state-of-the-art and recent technological advances. *Bioengineering* 2020;7(2):43; doi: 10.3390/bioengineering7020043
43. Rocamora I, Wagland ST, Villa R, et al. Dry anaerobic digestion of organic waste: A review of operational parameters and their impact on process performance. *Bioresour Technol* 2020;299:122681; doi: 10.1016/j.biortech.2019.122681

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