Methane potential of fruit and vegetable waste: an evaluation of the semi-continuous anaerobic mono-digestion

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To cite this article: Thiago Edwiges, Laercio Mantovani Frare, João Henrique Lima Alino, Jin Mi Triolo, Xavier Flotats & Mônica Sarolli Silva de Mendonça Costa (2018): Methane potential of fruit and vegetable waste: an evaluation of the semi-continuous anaerobic mono-digestion, Environmental Technology, DOI: 10.1080/09593330.2018.1515262

To link to this article: https://doi.org/10.1080/09593330.2018.1515262

Accepted author version posted online: 22 Aug 2018.

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Methane potential of fruit and vegetable waste: an evaluation of the semi-continuous anaerobic mono-digestion

Abstract

The anaerobic digestion (AD) of a high diversity blend of fruit and vegetable waste (FVW) generated in tropical conditions as a single substrate was performed. A continuously stirred tank reactor (CSTR) operated in semi-continuous regime was used for AD. The reactor performance was monitored with gradually increasing organic loading rates (OLRs) from 0.5 gVS L\(^{-1}\) d\(^{-1}\) up to 5.0 gVS L\(^{-1}\) d\(^{-1}\). The biochemical methane potential (BMP) of FVW determined by batch bottles was 360 L\(_N\) CH\(_4\) kg\(_{VS}\)^\(-1\), with a biodegradability of 79%. A stable pH with an adequate level of buffering capacity was observed during the entire experiment. Methane yield indicated the best performance at an OLR of 3.0 gVS L\(^{-1}\) d\(^{-1}\), with 285 L\(_N\) CH\(_4\) kg\(_{VS}\)^\(-1\) added, reaching 79% of BMP. At an OLR over 3.0 gVS L\(^{-1}\) d\(^{-1}\) accumulation of volatile fatty acids (VFA) was detected; in particular, propionic acid was monitored, and a decreased methane yield was detected. Biogas production rate was 1.55 L\(_N\) L\(^{-1}\) d\(^{-1}\) and showed linear increase according to increases in the OLR.

Keywords: biogas; BMP; biodegradability; CSTR; organic loading rate.

1. Introduction

Around 173 million inhabitants live in the urban perimeter of Brazil. Regarding the municipal solid waste (MSW) generated in the Brazilian urban area, less than 10% is properly treated or recycled and only 53% of it is disposed in adequate sanitary landfills [1]. Controlling the use of non-regulated landfills is still a challenge in the country and the lack of drainage systems for gases and leachates in these areas represents high environmental impact [2].

Due to the adequate climate and water availability, tropical countries are responsible for an expressive amount of fruits and vegetables. Brazil occupies the
leading position of this market, being the largest fruit and vegetable producer in the world [3]. To allow the commercialization of farm products, the Brazilian government has been implementing several public wholesale markets since 1970; nowadays, more than 60 of them are operating in all regions of the country [4].

As fruit and vegetable waste (FVW) has high content of water and rapid hydrolysable sugars, negative impacts such as strong odour, leachate production, and disposal costs are often reported [5, 6]. Anaerobic digestion (AD) of organic waste is a widely applied technology, and its main beneficial properties include the ability to treat high moisture-containing biomasses and small-scale applicability. In recent years, AD has been gaining more appeal, and its application has been emerging noticeably due to the increasing demand for renewable energy [7].

The operation of mono-digestion using FVW as a single substrate is often reported as an unstable process because of simple sugar degradation, volatile fatty acids (VFA) accumulation, and subsequently, fast system acidification. The AD of FVW used as a single substrate has been limited to an organic loading rate (OLR) under 3.5 gVS L$^{-1}$ d$^{-1}$ [8, 9, 10]. Several studies have described the AD of FVW [10, 11, 12], but only a few have used FVW as a single substrate. Alvarez and Lidén [13] and Jiang et al. [9] reported the failure of AD using only FVW due to VFA accumulation and subsequent pH reduction. Strategies used in these studies to promote a more stable digestion include the use of co-substrates and a solution of trace elements. To contribute to adequate chemical characteristics and avoid a high content of simple sugars, co-digestion with animal manure, sewage sludge, and other organic co-substrates has been used often. Unfortunately, due to logistical and economic issues, it is not always possible to guarantee suitable amounts of co-substrates as a feedstock [14].
The adoption of two-stage AD reactors has also been reported as a strategy to improve the process stability of substrates rich in simple sugars, as they allow the buffering of the OLR in the first stage and a more constant feeding rate in the methanogenic second stage. However, more complex and expensive treatment plants are required for this sort of operation. Ganesh et al. [8] and Shen et al. [15] evaluated the AD of FVW in both single-stage and two-stage reactors and reported the advantages of single-stage digestion due to simpler operation requirements, higher methane yields, and better economic benefits.

A better solution for preventing acidification effects may be the use of a well-balanced mixture of FVW, increasing the diversity of fruit and vegetable types in the substrate, therefore avoiding the use of those rich in simple sugars [14]. Thus, the aim of this study was to perform the anaerobic mono-digestion of a well-diversified blend of FVW generated in tropical conditions by applying different organic loading rates to evaluate system performance.

2. Materials and methods

2.1 Feedstock and seeding sludge

The mixture of FVW used as a substrate was collected from a municipal wholesale market (Foz do Iguaçu, Brazil). The market generates around 2,800 kg d\(^{-1}\) of solid waste, with 85% of its organic content being sent to landfill weekly. A representative sample of 20 L was collected at 6 different points of the waste container after a week (November 2016) and before its final destination. In total, 33 types of fruits and vegetables composed the mixture and qualitative composition indicated that 48% were fruit and 52% were vegetable waste based on the total amount of registered types. High diversity in the mixture was observed, as no type of fruit or vegetable represented more than 7% (wet basis) of the total amount (Figure 1).
Each type of collected fruits and vegetables was weighed and the mixture was grinded without water addition to preserve the sample characteristics, with particle size under 10 mm. The sample was then frozen at –18 °C, and the amount needed to feed the digester for one week was thawed and stored at +4 °C [16].

(Figure 1 near here)

As mesophilic biogas plants are not common in Brazil, the inoculum used for batch and semi-continuous tests was prepared with a mixture of two digestates (one from a biogas plant processing swine manure and cattle manure) and raw cattle manure, from the western region of the state of Paraná/Brazil. Maintenance of the biological activity of inoculum was performed by weekly feeding with a mixture of substrates at an OLR of 0.5 gVS L\(^{-1}\) d\(^{-1}\) [17]. Feeding was interrupted one week before the inoculum was used to respect the degassing period. Further information on the maintenance and composition of the inoculum is described in Edwiges et al. [18].

2.2 Batch assay

Biochemical methane potential (BMP) of the FVW mixture was determined in batch bottles (200 mL) coupled to eudiometer-graduated tubes (300 mL) and expressed in L\(_N\) CH\(_4\) kg VS\(^{-1}\) added. BMP of the mixture was determined to compare the batch and semi-continuous potential. The biological activity of the inoculum was assessed using microcrystalline cellulose as a reference sample (Sigma-Aldrich, 20μ diameter).

The solid content in batch bottles was kept according to the guidelines proposed by VDI 4630 (2006) to provide standardized results. The average value for total solids (TS) in the mixture (inoculum + substrate) contained in the batch bottles was around 4.1%; volatile solids (VS) related to inoculum was 1.9% average and the inoculum-to-substrate ratio based on VS was greater than 2. Nitrogen gas was flushed into each bottle to expel its oxygen content and temperature was controlled at 37 ± 2 °C by a
regulated water bath. Biogas production was daily registered until it represented less than 1% of accumulated biogas [16].

The biodegradability of FVW was evaluated indirectly through the relation between theoretical BMP (TBMP) and measured BMP. A detailed method for the estimation of TBMP is found in Triolo et al. [19].

2.3 Reactor design and operational conditions

Semi-continuous AD was performed in a continuously stirred tank reactor (CSTR) (B Braun Biotech–Biostat B) with double-walled glass and a 4 L working volume (Figure 2). Mesophilic temperature was controlled at $37 \pm 2 \, ^\circ C$ by a thermostatically regulated water bath, and stirring speed was kept at $60 \, \text{min}^{-1}$ to ensure adequate mixing and degassing. The anaerobic reactor was filled with 75% of pre-cultivated seeding sludge (as described in Section 2.2) and 25% of distilled water. Digestate feeding and discharging were carried out on a daily basis. For acclimatization of the microbial population to the feeding substrate, the digester was operated in a batch system for two weeks with $\frac{\text{VS}_{\text{FVW}}}{\text{VS}_{\text{inoculum}}} = 0.1$. Biogas volume was measured through an inverted beaker filled with an acid sealing solution ($\text{pH} < 2.0$).

(Figure 2 near here)

The initial OLR of $0.5 \, \text{gVS L}^{-1} \, \text{d}^{-1}$ was then used for 20 days during the start-up phase, with a hydraulic retention time (HRT) of 40 days [15]. Afterwards, the HRT was decreased to 30 days and substrate was fed in OLR’s of $0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0$ and $5.0 \, \text{gVS L}^{-1} \, \text{d}^{-1}$ with a transitional slope, i.e. the increased amount of VS added per day during 5 days, of $0.1 \, \text{gVS L}^{-1} \, \text{d}^{-1}$, except for the last OLR, when $0.2 \, \text{gVS L}^{-1} \, \text{d}^{-1}$ was used (Figure 4c). As VDI 4630 [16] suggest increasing the loading rate as soon as methane production is constant for at least four days (empirical value), this strategy was adopted. Additionally, the statistical coefficients of variation ($C_{\text{var}}$) of daily methane
production for each OLR was introduced to determine the ability of digestion system for new-fed substrate through the variability of data [10, 15]. The OLR was then changed whenever $C_{\text{var}}$ was under 10%.

The OLR employed in this study was based on the VS of fresh feedstock. Distilled water was used to prepare the initial feeding loads to maintain the HRT constant while varying the OLR. Thus, it was possible to evaluate the methane potential of only FVW with the same operational conditions, avoiding the effect of any co-substrates. For the last OLR, the system was operated with an HRT of 24 days due to the higher feeding rate needed to achieve 5.0 gVS L$^{-1}$ d$^{-1}$.

Biogas was drained using an outlet pipe at the top of the reactor, and volumetric production was daily measured through a graduated test tube, where an acid solution was used as a sealing liquid. The measured volume was corrected to dried biogas at normal temperature and pressure conditions [16].

2.4 Analytical methods and process monitoring

Total solids (TS), volatile solids (VS), pH, total alkalinity (TA), crude lipid, and total Kjeldahl nitrogen (TKN) were determined according to the standard methods [20]. Crude protein was estimated by multiplying TKN by 6.25 [21]. Partial alkalinity (PA), intermediate alkalinity (IA) and volatile acids (VA) in the digestate were determined according to Ripley et al. [22] and DiLallo and Albertson [23] to evaluate the monitoring process. The VA was determined by titration to a pH endpoint of 5.75 in order to express the ratio between total alkalinity/volatile acids (VA:TA), which can express the buffering contribution of bicarbonate during AD when it is under 0.3.

The VFA of the digestate was detected using high-performance liquid chromatography in a Shimadzu-2010 system equipped with an AminexHPX-87H column (300 mm long and 4.6 mm internal diameter BioRad) and an ultraviolet detector
with a diode arrangement, based on the methodology proposed by Lazaro et al. [24]. Methane concentration was determined by gas chromatography (Perkin Elmer-Clarus 680) using a thermal conductivity detector and a 30 m long packed Plot Q column packed with 0.32 mm internal diameter. Helium was used as carrier gas with a flow rate of 30 mL min\(^{-1}\).

Cellulose, hemicellulose, and lignin were determined through neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) [25]. Non-lignocellulosic carbohydrate was estimated by the difference between 100 and the sum of proteins, lipids, water, ashes, and lignocellulose. Trace elements of FVW and digestate were measured through atomic absorption spectrometry (Digimed DM-62) after nitro-perchloric digestion.

3. Results and discussion

3.1 Substrate and inoculum characteristics

The concentration of solids in the FVW mixture was similar to the ones found in the literature [8, 12], showing high values for moisture and volatile compounds (TS: 129 g kg\(^{-1}\); VS: 121 g kg\(^{-1}\)). These characteristics indicate the potential for biological treatment, fitting AD for waste treatment and bioenergy production. However, pH was 3.9 (Table 1), which may indicate limitation of AD due to methanogenesis.

(Table 1 near here)

Organic compounds were similar to those reported by Shen et al. [15] and Wang et al. [10]. Non-lignocellulosic carbohydrates were the most common among organic components (55.2%), indicating the potential for a fast conversion of organic matter into biogas. Regarding lignocellulose content (26.3%), the characteristics were more similar to those green grasses when compared to cellulosic biomass, e.g., agricultural
residues and wild plants [19], showing its potential for high methane production yields through AD.

The inoculum characteristics were TS 5.8 ± 0.1%, VS 62.3 ± 1.3% of TS, and the pH was of 8.3 ± 0.1. These main characteristics indicate an adequate level of microorganisms indicated by VDI 4630 [16] and a slightly alkaline medium. Total alkalinity was of 8.900 ± 94 mg CaCO$_3$ L$^{-1}$.

3.2 Biochemical methane potential

BMP measured from batch bottles was 360 ± 2 L$_N$ CH$_4$ kg$_{VS}^{-1}$ added, similar to that found by Jiang et al. [9] of 350 L$_N$ CH$_4$ kg$_{VS}^{-1}$ added while evaluating the AD of a vegetable mixture composed by beans, corn, carrots, and broccoli. Lin et al. [5] reported lower BMP of 300 L$_N$ CH$_4$ kg$_{VS}^{-1}$ added of a more diversified mixture of fruits and vegetables composed of cabbages, carrots, lettuces, apples, bananas, pears, and watermelons. Even so, the BMP of FVW was relatively high when compared to other organic substrates, such as 243, 303, and 268 L CH$_4$ kg$_{VS}^{-1}$ added of dairy manure [26], swine manure [27] and food waste [28], respectively.

The estimation of TBMP through chemical composition resulted in 456 L$_N$ CH$_4$ kg$_{VS}^{-1}$. Thus, biodegradability estimated by the relation between BMP/TBMP was 79%, which was remarkably higher than the values for other types of organic substrates. Triolo et al. [19] reported a 68% average of biodegradability for vegetable biomass, such as grass and wheat straw, and a 49% average for animal biomass such as cattle and pig manure. Cumulative methane (Figure 3) showed a fast conversion of FVW into biogas, in which more than 90% of organic matter was degraded in the first 5 days of the batch test. This fast methane production of FVW might be related to the organic composition, which mainly contained non-lignocellulosic carbohydrates (52.2%).

(Figure 3 near here)
3.3 Biogas production rate and methane yield

During the start-up period, biogas production rate was between 0.20 ± 0.02 L\textsubscript{N} L\textsuperscript{-1} d\textsuperscript{-1} (Figure 4a), with slightly lower production during the first OLR (0.5 gVS L\textsuperscript{-1} d\textsuperscript{-1}) (phase I) as a result of the HRT difference, which was of 40 days and 30 days, respectively. Later, the biogas rate increased according to a subsequent increase in OLR from 1.0 to 5.0 gVS L\textsuperscript{-1} d\textsuperscript{-1}, achieving 1.55 ± 0.12 L\textsubscript{N} L\textsuperscript{-1} d\textsuperscript{-1} (Table 2). This productivity was much higher when compared to other types of substrate, such as animal manure. Negral et al. [29] reported biogas productivity of only 0.3 L L\textsuperscript{-1} d\textsuperscript{-1} using raw dairy manure as a substrate at an OLR of 2.3 gVS L\textsuperscript{-1} d\textsuperscript{-1}. However, when using the liquid fraction of dairy manure, Rico et al. [30] reported biogas productivity similar to the one found in this study (1.47 L L\textsuperscript{-1} d\textsuperscript{-1}), as similarly to FVW, the liquid fraction of dairy manure presents high content of easily biodegradable compounds.

Statistical C\textsubscript{var} were under 10% of each OLR (Table 2), indicating data homogeneity during each operational phase. The highest CV variation was obtained until day 62, corresponding to an OLR of 1.5 gVS L\textsuperscript{-1} d\textsuperscript{-1} (phase III), as bacterial activity was being adjusted to operational conditions such as type of substrate and increasing OLR at the beginning of AD. Similarly, methane concentration in the biogas presented the lowest values at the beginning of the process, due to the low content of soluble organic matter in the inoculum.

Daily methane yield ranged from 151 ± 12 L\textsubscript{N} CH\textsubscript{4} kg\textsubscript{VS}\textsuperscript{-1} to 285 ± 10 L\textsubscript{N} CH\textsubscript{4} kg\textsubscript{VS}\textsuperscript{-1} added (Table 2), showing the best efficiency values at an OLR of 3.0 gVS L\textsuperscript{-1} d\textsuperscript{-1} (phase VI). Di Maria and Barratta [12] reported a similar methane yield of 236 L\textsubscript{N} CH\textsubscript{4} kg\textsubscript{VS}\textsuperscript{-1} during the AD of a mixture of organic substrate composed of potatoes (55%).
FVW (28%), bread (5%), paper (2%), and pasta (10%) at an OLR of 2.8 gVS L\(^{-1}\) d\(^{-1}\) and HRT of 35 days. A higher methane yield of 352 L\(_N\) CH\(_4\) kg\(_{VS}^{-1}\) was reported by Jiang et al. [9] while evaluating AD of FVW at an OLR of 3.0 gVS L\(^{-1}\) d\(^{-1}\) and a HRT of 26 days. The substrate used was composed of beans (75%), baby corn (19%), carrots (3%), and broccoli (3%) and a solution of trace elements was weekly added proportionally to 1 mL L\(^{-1}\) to achieve process stability. These studies highlight the limitations of AD with FVW as a single substrate concerning the OLR.

After an OLR of 3.5 gVS L\(^{-1}\) d\(^{-1}\) (phase VII) methane yield showed a constant decrease, starting on day 102 (Figure 4a). This behaviour might indicate the load limit of the anaerobic mono-digestion of FVW concerning conversion efficiency. Even with the downward trend starting on phase VII, the operation was kept until an OLR of 5.0 gVS L\(^{-1}\) d\(^{-1}\) (phase IX) as the biogas rate showed linear growth (R\(^2\) 0.94) with the increase in OLR. Phase IX presented methane yield of 198 ± 17 L\(_N\) CH\(_4\) kg\(_{VS}^{-1}\) added, this value being 21% lower than the one for the best production achieved and therefore, the AD process was interrupted at day 143. However, data on biogas production rate can provide useful information on the operational planning of biogas plants, as the system showed the ability to withstand the higher OLR.

The methane yield produced from CSTR achieved an average of 62% of measured BMP determined in batch bottles. The best performance was obtained at an OLR of 3.0 gVS L\(^{-1}\) d\(^{-1}\) (phase VI), in which 79% of BMP was achieved. Methane content ranged from 45% to 65% until day 62, and from 67% to 75% until day 133 (Table 2). During phase IX, when the OLR increased from 4.0 to 5.0 gVS L\(^{-1}\) d\(^{-1}\) and the HRT consequently reduced from 30 to 24 days, a drop in the methane content was observed. This can initially indicate inhibition in the methanogenic activity, as an accumulation of VFA was equally observed during this phase. However, as stated by
Rico et al. [31], increases in OLR and decreases in HRT may also contribute to lower methane content in the biogas. Furthermore, the higher methane content in the biogas between the OLR of 2.0 and 4.0 gVS L$^{-1}$ d$^{-1}$ was also associated to high alkalinity availability inside the reactor (Figure 4b), which is responsible for withholding carbon dioxide in its dissolved forms, i.e. H$_2$CO$_3$, HCO$_3^-$, CO$_3^{2-}$.

### 3.4 Digestate characteristics

During the digestion process, the pH of the digestate was in the range of 7.9 and 8.3 (Table 2), not showing a tendency to acidification with the increase of OLR. The concentration of total VFA in the digestate ranged from 22 to 390 mg L$^{-1}$, along with an increase from 0.5 to 3.0 gVS L$^{-1}$ d$^{-1}$ in the OLR (Table 2). Thereafter, a considerably later increase was observed, with VFA reaching 1,781 mg L$^{-1}$ at the OLR of 5.0 gVS L$^{-1}$ d$^{-1}$ (Figure 5a), due to the high soluble carbohydrates composition of FVW.

The accumulation of VFA after OLR of 3.0 gVS L$^{-1}$ d$^{-1}$ is a sign of instability, as we can see in the reduction of biogas yield and methane concentration after this period. The propionic acid content represented most of the VFA content, showing an average of 51%, reaching 63% for the OLR at 5.0 gVS L$^{-1}$ d$^{-1}$ (Figure 5b). Shen et al. [15] and Wang et al. [10] have similarly reported accumulation of VFA during AD of FVW, mainly as propionic acid.

(Figure 5 near here)

Even with VFA accumulation, the pH was kept in a slightly alkaline range, mainly because of its buffering capacity of the bicarbonate/carbon dioxide equilibrium [5]. Total alkalinity was sufficiently high during AD, with a concentration above 2.6 g CaCO$_3$ L$^{-1}$ for OLR values ranging from 0.5 to 2.5 gVS L$^{-1}$ d$^{-1}$ (Figure 4b). After an OLR of 3.0 gVS L$^{-1}$ d$^{-1}$, total alkalinity was above 3.5 g CaCO$_3$ L$^{-1}$, increasing to 6.4 g CaCO$_3$ L$^{-1}$ until the end of the operation. This can be mainly explained by the alkalinity
produced by ammonium bicarbonate, whose ammonia (NH$_3$) released from the
degradation of proteins and amino acids reacted with carbon dioxide and water,
providing alkalinity to the system (Equation 1).

$$\text{NH}_3 + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_4\text{HCO}_3$$

During the entire experiment, both IA:PA and VA:TA ratios were consistently
below 0.3 (Table 2), showing the buffering contribution of bicarbonate and indicating a
scenario of stable operation. However, as the VFA started to accumulate after an OLR
of 3.5 gVS L$^{-1}$ d$^{-1}$ (Figure 4c), these ratios did not seem to reflect its initial inhibition
aspect. The concentration of acetic acid and butyric acid of 2,400 and 1,800 mg L$^{-1}$,
respectively, were not reported in the literature as inhibitors. On the other hand,
propionic acid concentration of 900 mg L$^{-1}$ resulted in the significant inhibition of the
methanogens. As stated by Wang et al. [32], the propionic conversion rate to methane is
slower than the acetic and butyric acids. Thus, propionic acid accumulation results in
methanogenic inhibition in concentrations lower than those of other organic acids.

(Figure 5 near here)

The concentration of acetic and butyric acid in this study was below 500 mg L$^{-1}$
during the entire experiment, but the concentration of propionic acid was considerably
high (1,113 mg L$^{-1}$ at an OLR of 5.0 gVS L$^{-1}$ d$^{-1}$), indicating the degradation of
propionic acid had is slowed down, which was probably due to accumulation of H$_2$, as
degradation of propionic acid is thermodynamically unpreferable with H$_2$ accumulation.
The accumulation of propionic acids can also be affected by the lack of trace elements,
as anaerobic biochemical reactions, including the conversion of organic acids into
methane, requires essential enzymes containing metals such as cobalt, nickel, iron, and
zinc molybdenum and tungsten [9]. However, as can been see in Table 3, the content of
the metals was sufficient, showing that mono-digestion of FVW does not require trace
metal supply. This was observed by Williams et al. [33], who reported biogas production stimulation by adding extra 0.59 mg L\(^{-1}\) of nickel, while its concentration on digestate was of 14.8 mg L\(^{-1}\). Ortner et al. [34] also reported only 62% of Ni and Co bioavailability of total concentration while investigating the influence of trace elements on AD of slaughterhouse waste.

(Table 3 near here)

4. Conclusions

The results of this study have demonstrated that mesophilic anaerobic monodigestion of a highly diverse mixture of fruits and vegetables is possible. These results highlight the potential use of a biomass source that is not so common used in developing countries. A stable process operation was observed at an OLR of up to 5 gVS L\(^{-1}\) d\(^{-1}\), with highest rate of biogas production achieved at 1.55 L±0.12 L\(^{-1}\) d\(^{-1}\) and HRT of 24 days. However, the highest efficiency rate regarding the specific methane yield was obtained at an OLR of 3 gVS L\(^{-1}\) d\(^{-1}\), with 285±26 L\(_{N}\) CH\(_4\) kg\(_{VS}\)\(^{-1}\). VFA accumulation, especially propionic acid with concentrations above 1,000 mg L\(^{-1}\) was detected in the last OLR, indicating the operational limits of the anaerobic monodigestion process for this type of biomass.

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Improvement of fruit and vegetable waste anaerobic digestion performance and stability


FIGURE CAPTIONS

• Figure 1. Qualitative composition of FVW (%) used as substrate.

• Figure 2. Experimental set up scheme.

• Figure 3. Cumulative methane production of FVW. Methane production from FVW was expressed subtracting the endogenous production related to inoculum.

• Figure 4. Biogas rate (↔) (left) and methane yield (→) (right) during different operational phase (a); total alkalinity (●) (left) and pH of digestate (○) (right) during different operational phases (b); start period of each OLR tested during the operational phase (—) (left) and methane content of biogas (○) (right) during each operational phase (c). OLR was 0.5, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0 g SV L⁻¹ d⁻¹ for operational phase I, II, III, IV, V, VI, VII, VIII, IX, and X, respectively.

• Figure 5. Concentration of VFA (a) and percentage of each VFA related to total concentration (b) during different operational OLR.
Figure 1.

Figure 2.
Figure 3.
Figure 4.
Figure 5.
Table 1. Composition of fruit and vegetable waste used as a substrate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (g kg$^{-1}$)</td>
<td>129 (±17)$^a$</td>
</tr>
<tr>
<td>Volatile solids (g kg$^{-1}$)</td>
<td>121 (±5)</td>
</tr>
<tr>
<td>Density (kg L$^{-1}$)</td>
<td>1.03 (±0.02)</td>
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<tr>
<td>pH</td>
<td>3.9 (±0.03)</td>
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<tr>
<td>Total Kjeldahl nitrogen (% dry weight)</td>
<td>2.2 (±0.1)</td>
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<tr>
<td>Crude protein (%VS)</td>
<td>15.8 (±0.6)</td>
</tr>
<tr>
<td>Crude lipid (%VS)</td>
<td>2.7 (±0.1)</td>
</tr>
<tr>
<td>Cellulose (%VS)</td>
<td>11.1 (±0.1)</td>
</tr>
<tr>
<td>Hemicellulose (%VS)</td>
<td>11.4 (±0.3)</td>
</tr>
<tr>
<td>Lignin (%VS)</td>
<td>3.8 (±1.4)</td>
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<tr>
<td>Non-lignocellulosic carbohydrates (%VS)</td>
<td>55.2 (±0.8)</td>
</tr>
<tr>
<td>Co (mg L$^{-1}$)</td>
<td>1.35 (±0.27)</td>
</tr>
<tr>
<td>Cu (mg L$^{-1}$)</td>
<td>&lt; D.L.</td>
</tr>
<tr>
<td>Fe (mg L$^{-1}$)</td>
<td>105.69 (±16.20)</td>
</tr>
<tr>
<td>Mo (mg L$^{-1}$)</td>
<td>&lt; D.L.</td>
</tr>
<tr>
<td>Ni (mg L$^{-1}$)</td>
<td>0.27 (±0.001)</td>
</tr>
<tr>
<td>Se (mg L$^{-1}$)</td>
<td>&lt; D.L.</td>
</tr>
<tr>
<td>Zn (mg L$^{-1}$)</td>
<td>5.73 (±1.29)</td>
</tr>
</tbody>
</table>

$^a$Parentheses represent standard deviations (SD). D.L.: detection limit.

Table 2. Summary of anaerobic digestion performances

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLR (g VS L$^{-1}$ d$^{-1}$)</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td>3.5</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Biogas rate (L N$^{-3}$ d$^{-1}$)</td>
<td>0.17±0.02</td>
<td>0.33±0.02</td>
<td>0.43±0.04</td>
<td>0.73±0.05</td>
<td>0.83±0.04</td>
<td>1.19±0.11</td>
<td>1.28±0.07</td>
<td>1.43±0.07</td>
<td>1.55±0.12</td>
</tr>
<tr>
<td>Methane yield (L CH$_4$ kg VS$^{-1}$)</td>
<td>151±12</td>
<td>214±11</td>
<td>164±16</td>
<td>247±14</td>
<td>228±10</td>
<td>285±26</td>
<td>262±14</td>
<td>267±13</td>
<td>198±17</td>
</tr>
<tr>
<td>CV (%)</td>
<td>10</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>CH$_4$ Content (%)</td>
<td>43±0.2</td>
<td>65±2.3</td>
<td>64±2.9</td>
<td>67±0.3</td>
<td>69±0.5</td>
<td>72±1.7</td>
<td>72±2.6</td>
<td>75±0.1</td>
<td>64±3.7</td>
</tr>
<tr>
<td>pH</td>
<td>8.1±0.1</td>
<td>7.9±0.1</td>
<td>7.8±0.1</td>
<td>7.9±0.1</td>
<td>7.9±0.1</td>
<td>8.0±0.1</td>
<td>8.3±0.2</td>
<td>8.1±0.1</td>
<td>8.1±0.3</td>
</tr>
<tr>
<td>Total VFA (mg L$^{-1}$)</td>
<td>22±1</td>
<td>247±9</td>
<td>77±2</td>
<td>285±12</td>
<td>338±21</td>
<td>390±21</td>
<td>983±25</td>
<td>970±29</td>
<td>1,781±42</td>
</tr>
</tbody>
</table>
Total alkalinity (mg L$^{-1}$) 3,744±291 3,272±155 2,875±126 2,678±42 2,806±120 3,572±271 4,745±379 5,604±480 6,434±801

IA:PA 0.10 0.13 0.12 0.09 0.13 0.13 0.14 0.13

VA:TA 0.15 0.18 0.15 0.10 0.13 0.12 0.09 0.11 0.10

OLR: organic loading rate; HRT: hydraulic retention time; CV: coefficient of variation related to biogas production; VS: volatile solids; VFA: volatile fatty acids; IA:PA: intermediate alkalinity to partial alkalinity ratio; VA:TA: volatile acids ratio.

Table 3. Summary of trace elements in digestate for each operational phase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Operational phase</th>
<th>Optimal values reported in literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg L$^{-1}$</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Cu</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Fe</td>
<td>44.6</td>
<td>35.3</td>
</tr>
<tr>
<td>Ni</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Zn</td>
<td>5.3</td>
<td>3.7</td>
</tr>
</tbody>
</table>