Effects of Experimental Parameters on Methane Production and Volatile Solids Removal from Tomato and Pepper Plant Wastes

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In Mexico, protected agriculture generates large amounts of tomato and pepper plants residues (TPW and PPW, respectively). Given the limited information on methane production from anaerobic digestion of these wastes, this study aimed to determine the effects of the substrate/inoculum (S/I) ratio, temperature, and total solids content on methane production and volatile solids (VS) removal by two subsequent batch experiments (Experiments A and B). Experiment A was performed to evaluate the substrate/inoculum ratios of 0.5, 1.0, and 2.0 at room temperature (22 ± 4.5 °C). Based on the best methane yield from experiment A, a new experiment was established (Experiment B) using only tomato wastes, where temperature was kept at 29 °C and 39 °C. The total solids content was analyzed depending on the S/I ratio used. For both substrates, an S/I ratio of 0.5 was the most appropriate for methane production. The temperature had a positive effect on volatile solids removal and methane yield. In contrast, the total solids content (% TS) only had a positive effect on methane production. To the authors' knowledge, this is the first study evaluating the effect of the S/I ratio on methane production from tomato and pepper plant wastes.

Keywords: Anaerobic digestion; Agriculture waste; Biogas

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INTRODUCTION

In recent years, studies on biogas production by anaerobic digestion have focused on the use of residual biomass as substrates, which allows the substrate to be properly disposed of while obtaining energy at the same time (Al Seadi *et al.* 2013). To date, the potential for biogas production has been evaluated from the agricultural wastes of corn, sorghum, wheat, rice, and sugarcane, among others, due to the wide distribution of production of these crops in many countries (Zheng *et al.* 2014; Mao *et al.* 2015). However, other crops at the regional level highly influence waste generation. In Mexico, tomato and pepper production represents a substantial share of the national economy, because these crops are the main crops produced by protected agriculture (INIFAP 2017).

Most studies in which wastes from tomato and pepper crops are used for biogas production focus on fruit use. Thus, the information available from the plant wastes of these crops is limited.

To improve the biogas production yield in batch tests, several authors mention the importance of evaluating various substrate/inoculum ratios (S/I) (Angelidaki *et al.* 2009). An adequate S/I ratio favors the balance between the different groups of microorganisms carrying out the hydrolysis and methanogenesis stages of anaerobic digestion, thus using the substrate more efficiently (Eskicioglu and Ghorbani 2011). Furthermore, knowing the optimal S/I ratio prevents problems of methanogenic inhibition due to the accumulation of volatile fatty acids (VFAs) (Raposo *et al.* 2011).

The effect of the S/I ratio has been evaluated for various substrates including kitchen waste (Neves *et al.* 2004; Xu *et al.* 2012; Kawai *et al.* 2014; Haider *et al.* 2015), agricultural waste (Raposo *et al.* 2006), industrial food waste (Pellera and Gidarakos 2016), and livestock waste (Córdoba *et al.* 2017).

The most recommended S/I ratios range between 0.5 and 1.0, negatively affecting methane production yield as the S/I ratio increases (Raposo *et al.* 2006; Zhou *et al.* 2011). However, Pellera and Gidarakos (2016) demonstrated that the amount of inoculum depends on the substrate characteristics; therefore, tests are needed to determine the S/I ratio at which the maximum biogas production yield is obtained for each feedstock.

Temperature and total solids (TS) content are other important control parameters that affect the anaerobic digestion process (Safari *et al.* 2018). In general, a higher temperature causes an increase in chemical and biological reaction rates, improving substrate degradation efficiency (Chae *et al.* 2007; Choorit and Wisarnwan 2007). In contrast, an increase in the total solids content can cause an overloading of the digesters (Baserja 1984).

The production of methane from tomato plant residues has been studied either alone or in co-digestion (Jagadabhi *et al.* 2011; Akman *et al*. 2015; Li *et al.* 2016; Oleszek *et al.* 2016). Jagadabhi *et al*. (2011) focused on studying the reactor configuration to improve methane production, while Oleszek *et al*. (2016) evaluated the effect of plant silage. Akman *et al*. (2015) and Li *et al.* (2016) focused on evaluating various plant proportions with other substrates. Regarding the use of the pepper plant, different proportions of the plant were evaluated in co-digestion with cattle manure (Akman *et al*. 2015) and another study conducted by Guil-Guerrero *et al.* (2016) on the effect of silage in biogas production was studied through a predictive analysis. In all these studies, the temperature used was 35 °C to 37 °C, and methane production yields ranged between 130.3 mL/g VS and 415.4 mL/g VS. None of these studies assessed the variation of the S/I ratio, the temperature, or TS content. Therefore, the aim of the present study is to assess the effect of these variables on methane production and volatile solids removal from tomato and pepper agricultural wastes at lab scale.

EXPERIMENTAL

Materials

Feedstock and inoculum

The substrates used in this study consisted of the aerial part (stem and leaves) of tomato (TPW) and pepper (PPW) plant wastes collected at the end of the life cycle at the Agrifood Expo facilities located in the city of Irapuato, Guanajuato, Mexico. The tomato and pepper varieties were *Saladette* and *Lamuyo*, respectively.

The plants were dried under sunshine (19.9 °C ± 8.1 °C) for 15 d until 8 ± 3% moisture content was reached. The dried plant was milled in an agricultural hammer mill and stored at room temperature until use. Subsequently, a sample of 200 g was milled in a cereal and grain mill (SURTEK, Grupo Urrea Salamanca, Guanajuato, Mexico) and passed through a set of laboratory sieves (W.S. Tyler, Mentor, OH, USA). Samples whose particle size were between 0.85 mm and 1.68 mm were selected for the study considering previous tests developed at the lab (data not shown).

The inoculum used consisted of anaerobic sludge collected from a 1000-L geomembrane bag biodigester fed with a mixture of cow manure and water (7% to 10% TS, pH 6.83 ± 0.14), and operated at room temperature (19.9 °C ± 8.1 °C) with solid retention time of 7 d. The digester was installed in the experimental unit of the Laboratory of Technology for Sustainability, University of Guanajuato (Irapuato, Guanajuato, Mexico). The collected inoculum was degassed at room temperature (19.7 ± 7.0 °C) for 10 d. The TS and VS contents of the inoculum used were 12.17% ± 0.21% on a wet basis and 53.52% ± 0.02% on a dry basis, respectively.

Experimental Design

Two successive experiments were performed to evaluate methane production. Experiment A consisted in a 3 × 2 factorial design where the type of substrate (TPW and PPW) and S/I ratio (0.5, 1.0, and 2.0) at room temperature (22.0 °C ± 4.5 °C) were studied. The inoculum concentration was fixed at 13.0 ± 0.2 g.VS/L and the substrate concentration varied to obtain the desired S/I ratio (Table 1).

Table 1. Conditions for Each Treatment of Experiment A for Methane Production from TPW and PPW

Treatment	Substrate	S/I	Temperature (°C)	g $VS_{substrate}$/L	g $VS_{inoculum}$/L	% TS*
1A	TPW	0.5	22.0 ± 4.5	6.5	13.0	3.2
2A	TPW	1.0	22.0 ± 4.5	13.0	13.0	4.1
3A	TPW	2.0	22.0 ± 4.5	26.0	13.0	5.7
4A	PPW	0.5	22.0 ± 4.5	6.5	13.0	3.3
5A	PPW	1.0	22.0 ± 4.5	13.0	13.0	4.2
6A	PPW	2.0	22.0 ± 4.5	26.0	13.0	5.9

*TS contributed by both the inoculum and the substrate

For the second experiment, tomato substrate was utilized (experiment B). Treatments were divided into two groups based on the S/I ratio. This resulted in two factorial designs 2 × 2 that included as independent variables "temperature" and "% TS" in the mixture. The levels assigned for the variable “temperature” were 29 °C and 39 °C and the levels assigned for the variable “% TS” depended on the S/I ratio used in each treatment group. For the experimental design that included the treatments at an S/I ratio of 0.5, the levels of the “% TS” factor corresponded to 3.2 and 4.9, while in the experimental design that included the treatments at an S/I ratio of 1.0, the levels of %TS a factor of 4.1 and 6.1 were used. Note that independently of the S/I ratio used, at the low and high levels of "% TS", inoculum concentrations of 13.0 and 19.5 g VS/L were used, respectively. Both at an S/I ratio of 0.5 and 1.0, the high level of “% TS” corresponded to a 50% higher TS content in the mixtures compared to the low level (Table 2).

Table 2. Conditions for Each Treatment of Experiment B for Methane Production from TPW

Treatment	Substrate	S/I	Temperature (°C)	g $VS_{substrate}$/L	g $VS_{inoculum}$/L	% TS*
1B	TPW	0.5	29.0 ± 1.0	6.5	13.0	3.2
2B	TPW	0.5	29.0 ± 1.0	9.8	19.5	4.9
3B	TPW	0.5	39.0 ± 1.0	6.5	13.0	3.2
4B	TPW	0.5	39.0 ± 1.0	9.8	19.5	4.9
5B	TPW	1.0	29.0 ± 1.0	13.0	13.0	4.1
6B	TPW	1.0	29.0 ± 1.0	19.5	19.5	6.1
7B	TPW	1.0	39.0 ± 1.0	13.0	13.0	4.1
8B	TPW	1.0	39.0 ± 1.0	19.5	19.5	6.1

*TS contributed by both the inoculum and the substrate

To determine the significant differences in methane production between treatments carried out at room temperature (22 °C ± 4.5 °C) and those in which a controlled temperature (29 °C or 39 °C) was used, a 3 × 2 factorial design was used that included the variables S/I ratio (0.5 and 1.0) and temperature (22 °C ± 4 °C, 29 °C, and 39 °C). This statistical analysis only included those treatments in which TPW (1A and 2A) and inoculum concentration of 13.0 g VS/L (1B, 3B, 5B, and 7B) were used to perform an experimental comparison and validation of data.

The statistical analyses and significances were carried out by analysis of variance (ANOVA) with a P value of 0.05 using Statgraphics Centurion XVI (Statpoint Technologies, Inc., version 16.1.03, The Plains, VA, USA).

Batch Assays for Methane Production

The methane production assays were performed in serological bottles with total volume of 120 mL and working volume of 80 mL in a batch regime during a 30 d incubation period.

The substrate and inoculum were added to the serum bottles according to the experimental designs shown in Tables 1 and 2. The total solids content was adjusted to 80 mL with a mineral medium (Lara *et al.* 2014) whose composition per liter was: 4.8 g KH_2PO_4, 6.98 g K_2HPO_4, 6.0 g NH_4Cl, 0.1 g $MgCl_2 \cdot 6H_2O$, 0.02 g $CaCl_2$, 0.015 g $MnSO_4 \cdot 6H_2O$, 0.025 g $FeSO_4 \cdot 7H_2O$, 0.005 g $CuSO_4 \cdot 5H_2O$, and 0.125 mg $CoCl_2 \cdot 5H_2O$. No extra content of phosphorous was added to the mixture. The initial pH of the mixture was not adjusted because its value was close to a neutral pH. An endogenous control was included consisting of inoculum with only mineral medium.

For the mixtures prepared in each treatment, the TS and VS contents and pH were determined at the beginning and end of the incubation period. Each treatment was performed in duplicate.

Kinetics of Methane Production

To calculate the duration of the lag phase (h), the modified Gompertz equation (Eq. 1) (Pellera and Gidarakos 2016) was used; Eq. 1 was fitted with the software SigmaPlot 12.0 (Systat Software, Inc., San Jose, CA, USA),

$$B = M * exp\left\{- exp\left[\frac{R_m \cdot e}{M} * (\lambda - t) + 1\right]\right\} \quad (1)$$

where B is cumulative methane production during the test period (mL CH_4), M is the methane production potential (mL CH_4), R_m is the maximum methane production rate (mL CH_4/d), e = 2.718281828, λ is the lag phase time (h), and t is the incubation time (d).

Analytical Methods

The following analyses were performed for substrate characterization: the organic carbon content was determined by the loss-on-ignition method (Dean 1974), the structural carbohydrate content was determined by the method of Van Soest *et al.* (1991), the reducing sugar content was determined according to Ajani *et al.* (2011), nitrogen determination was performed using the Kjeldahl method, and the pH was measured using the method reported by Kang *et al.* (2014). The pH measurement of the samples from the biogas tests was carried out by shaking the sample manually; it was left to stand for 10 min and the supernatant reading was taken. Determination of the TS and VS contents and chemical oxygen demand (COD) by the closed reflux method was performed according to standard procedures (APHA 2005). The C/N ratio was determined by dividing the total organic carbon content by the total nitrogen content (Wang *et al*. 2013).

The volume of methane produced was determined by liquid displacement using a 4 M NaOH alkaline solution to absorb carbon dioxide (Drosg *et al.* 2013). The displaced volume was considered as equal to the methane production. The reactors were shaken manually at the time of gas measurement.

The presence of methane in the biogas was verified by gas chromatography for the detection of H_2, O_2, N_2, and CH_4, taking 500 µL of the gas present in the headspace of each bottle. This measurement was performed using a PerkinElmer Clarus 580 chromatograph (PerkinElmer, Shelton, CT, USA), with an Elite CG GS-MOSIEVE 52 capillary column (30 m × 0.53 mm × 50 µm) and a thermal conductivity detector (TCD). The temperatures of the injector, oven, and detector were 150 °C, 50 °C, and 200 °C, respectively. An argon pressure of 14 psi was used as mobile phase.

RESULTS AND DISCUSSION

Physicochemical Characterization

A similar value of C/N ratio was obtained for both TPW and PPW (19.9 and 19.7, respectively). According to the literature (Mao *et al.* 2015), the optimum range of the C/N ratio is from 20 to 30. An inhibition of bacterial activity due to ammonium accumulation or lack of nitrogen-promoting cell growth was not expected because C/N values close to those recommended for anaerobic digestion were obtained. Kang *et al.* (2014) reported a C/N ratio for the aerial part of the pepper plant to be 19.3, which is similar to that obtained in this report.

The VS content obtained for TPW and PPW was 75.9% and 75.8%, respectively. These values of VS were lower than the results of Akman *et al.* (2015) and Guil-Guerrero *et al.* (2016). These authors reported a VS value of 81% to 83% for these plants. This difference could be due to the fact that these studies considered the root part of the plant, which could contribute an additional amount of VS.

The cellulose, hemicellulose, and lignin contents for tomato plant were 28.6%, 8.4%, and 7.5% (based on dry weight), respectively. For pepper plant 19.4%, 13.8%, and 8.4% of cellulose, hemicellulose, and lignin, respectively, were obtained. Compared to other lignocellulosic substrates, such as wheat straw, rice, corn stubble, and grass, TPW and PPW had approximately half of cellulose and hemicellulose and similar lignin content (Li *et al.* 2013). These results showed that the aerial parts of tomato and pepper plants had lower carbohydrate contents available for fermentation and a substantial percentage of lignin, which could inhibit substrate biodegradation (Li *et al.* 2013; Zheng *et al.* 2014).

The content of reducing sugars for TPW was 1.7% while that for PPW was 4.6%. Therefore, the TPW had a lower reducing sugar content than the PPW, similar to that reported by Oleszek *et al.* (2016). These values might have influence on methane production.

Regarding the determination of COD, 788.0 and 860.6 mg COD/g VS were obtained for TPW and PPW, respectively. However, these values differed from those reported by Akman *et al.* (2015), who obtained 561 mg COD/g VS and 1154 mg COD/g VS for tomato and pepper vegetable wastes, respectively. In contrast, the COD reported for plant wastes, such as silage sorghum forages and wheat straw, is approximately 1.2 g COD/g VS, so the substrates used in this study had lower COD. The results from this study involved a lower methane production potential compared with other lignocellulosic substrates (Sambusiti *et al.* 2013). Further analyses of COD were performed on the treatments; however, statistical reproducibility was not achieved because of the data dispersion among same treatments. Instead, the VS content was used to establish the organic matter removal, which turned out much better for analysis and discussion of results. Table 3 shows the physicochemical characterization of TPW and PPW.

Table 3. Physicochemical Characterization of TPW and PPW

Parameters	TPW	PPW
C/N	19.86 ± 0.67	19.72 ± 3.32
TS (%)*	93.93 ± 0.32	91.74 ± 0.85
VS (%)**	75.89 ± 0.62	75.79 ± 0.10
Cellulose (%)**	28.57 ± 1.40	19.37 ± 1.41
Hemicellulose (%)**	8.42 ± 1.08	13.77 ± 1.01
Lignin (%)**	7.49 ± 1.13	8.43 ± 0.78
Reducing Sugars (%)**	1.70 ± 0.18	4.58 ± 0.07
COD (mg/g VS)	787.96 ± 26.97	860.60 ± 17.55
pH	6.60 ± 0.06	6.15 ± 0.04
* Wet basis percentage, **Dry basis percentage		

Experiment A

Experiment A consisted of evaluating the effect of the S/I ratio (0.5, 1.0, and 2.0) on methane production for substrates TPW and PPW at room temperature (19.7 ± 7.0 °C).

Fluctuations of T80 and lag phase

For experiment A, the time required to reach at least 80% of the total CH_4 production (T80, technical digestion time) generated during the 30 d incubation period was calculated. This parameter is an indicator of the methane production rate and has been used to compare the process performance according to the type of substrate and S/I ratio (Cheng and Zhong 2014; Pellera and Gidarakos 2016; Córdoba *et al.* 2017).

Figure 1 does not show the value of T80 for the S/I ratio of 2.0, because this condition inhibited methane production, as mentioned in the previous sections. The lowest T80 values were obtained from TPW.

Figure 1a shows that 80% of the total methane production was reached at 385 h at an S/I ratio of 0.5. However, the T80 increased 43% at an S/I ratio of 1.0. For PPW, the T80 increased 45% as the S/I ratio increased from 0.5 to 1.0 (Fig. 1b). This means that the S/I value affected the time required to reach 80% of production for both substrates. Therefore, using a higher feed load caused a delay in biogas production, which corroborated well with a study by Córdoba *et al.* (2017).

In addition, Rodriguez-Chiang and Dahl (2015) achieved faster methane production at an S/I ratio of 0.5 compared with the other S/I ratios evaluated (1.0, 1.25, and 2.0) using residual water derived from the production of microcrystalline cellulose as the substrate, which is consistent with what was obtained in this study.

The literature reported that a high proportion of inoculum increases the rate of biogas production because this condition decreases the time necessary to achieve enough growth of the methanogenic population in the inoculum (Raposo *et al*. 2006; Boulanger *et al*. 2012).

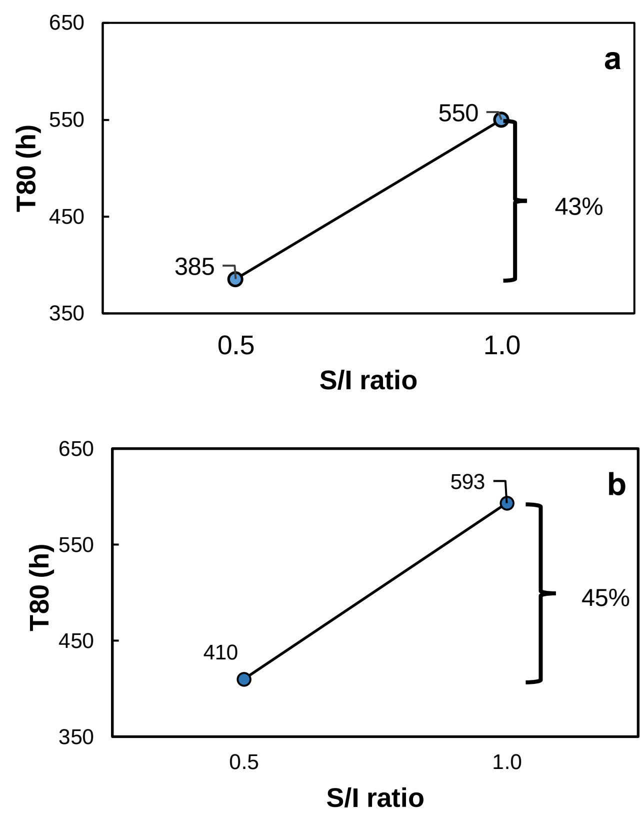

Fig. 1. The effect of S/I ratio from anaerobic digestion of TPW (a) and PPW (b) on technical digestion time (T80) of methane production

Through fitting the modified Gompertz equation, lag phase values were obtained for each of the methane production curves corresponding to treatments using S/I ratios of 0.5 and 1.0 (Table 4). Although the fit using the Gompertz equation is applied to curves in which the substrate is completely depleted mainly to calculate methane production potential, in this study, the fit was performed to calculate the duration of the lag phase. The R^2 value obtained for each curve (0.98 to 0.99) showed a good fit using the indicated model.

Table 4. Estimation of the Lag Phase (λ) and Determination Coefficient (R^2) at Different S/I Values from Gompertz Equation

Parameters	TPW		PPW	
S/I	0.5	1.0	0.5	1.0
λ	2.23	3.82	3.51	7.26
Adjusted R^2	0.99	0.99	0.99	0.98

The shortest duration of the lag phase was observed in treatments in which TPW was used; additionally, the lag phase increased as the inoculum amount decreased regardless of the substrate used.

Boulanger *et al.* (2012) and Kawai *et al.* (2014) reported similar outcomes. These authors mention that the duration of the lag phase is longer at higher S/I ratios, because there is a lower number of microorganisms available to degrade the organic matter present in the substrate, which leads to an accumulation of volatile fatty acids and inhibition of the growth of microorganisms. Furthermore, Kawai *et al.* (2014) described that, despite the eventual consumption of cumulative VFAs and pH recovery, the consumption rate of VFAs decreased as the S/I ratio was increased.

Influence of the S/I ratio on methane production

Figure 2 shows that the TPW had a higher production yield of CH_4/g VS than PPW, and the methane production yield was higher at an S/I ratio of 0.5 compared with the other two values evaluated (1.0 and 2.0) for both plants. The type of substrate and the S/I ratio significantly influenced the methane production ($p = 0.0001$ and $p = 0.0000$, respectively). Hence, the highest yield obtained was 166.2 mL CH_4/g VS, corresponding to treatment 1A, in which TPW and S/I of 0.5 were used. These results were consistent with those reported in the literature, stating that degradation was faster at lower S/I ratios, especially with substrates that are difficult to biodegrade, which is explained by the greater number of microorganisms present for substrate degradation as the S/I ratio decreases (Nielsen and Feilberg 2012; Rodriguez-Chiang and Dahl 2015).

At an S/I ratio of 2.0, for both TPW and PPW, a significant decrease in methane production was observed at 165 h (7 d). The pH of all the treatment mixtures remained neutral during the 30 d incubation period except for treatments 3A and 6A, in which the pH decreased to 6.1 and 6.4, respectively. This result was attributed to system overload, which agrees with other studies mentioning that the process becomes more susceptible to methanogenic inhibition at higher S/I ratios because of the accumulation of VFAs (Neves *et al.* 2004; Zhou *et al.* 2011; Rodriguez-Chiang and Dahl 2015).

Because the C/N ratio, the COD, and VS content in TPW and PPW were similar to each other, it cannot be attributed to these parameters that TPW performed better than PPW (either an S/I ratio of 0.5 or 1.0). Therefore, the variation in yield was mainly attributed to the content of structural carbohydrates present in these substrates, specifically the cellulose and hemicellulose content. PPW have 63.5% higher hemicellulose content than TPW, while TPWs have 47.5% higher cellulose content than PPW. Cellulose and hemicellulose are considered recalcitrant compounds that restrict hydrolysis during the first step of the anaerobic digestion process (Himmel *et al.* 2007). In a study by Li *et al.* (2018), they investigated the behavior of methane production from anaerobic mono-digestion of cellulose, hemicellulose, and lignin and showed that the methane potential of cellulose was higher than that of hemicellulose. Due to the higher cellulose content in TPW compared to PPW, this could explain the higher yield of methane obtained from TPW.

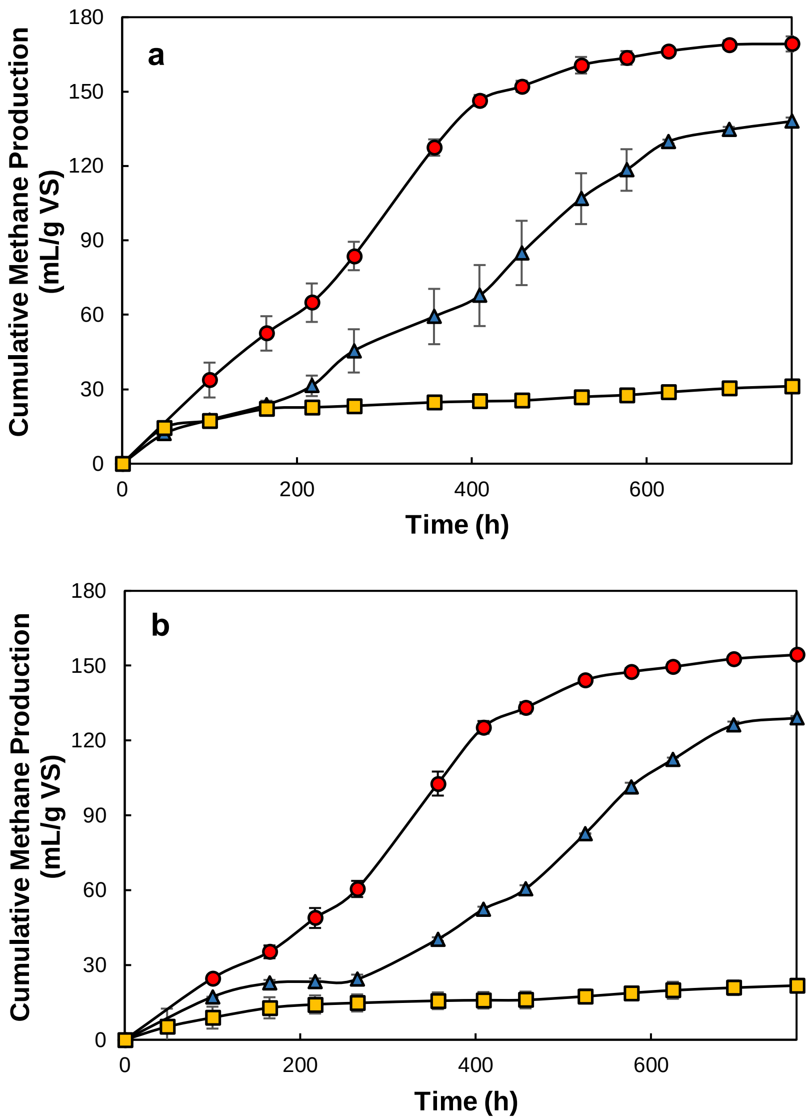

Fig. 2. Cumulative methane production (mL/g VS) from anaerobic digestion of TPW (a); and PPW (b) of experiment A: S/I ratio: 0.5 (●), 1.0 (▲), and 2.0 (■); error bars represent the standard deviation of each point (triplicates).

Influence of the S/I ratio on VS removal

Figure 3 shows both substrates TPW and PPW had the highest VS removal values at S/I 2.0, but PPW showed higher removal than substrate TPW. The type of substrate and the S/I ratio significantly influence the VS removal ($p = 0.0004$ and $p = 0.0015$, respectively). Therefore, the highest removal was 36% at S/I 2.0 using PPW (Treatment 6A).

The results from this study showed that the highest VS removal rate was obtained by using the highest S/I ratios corresponding to treatments in which methane production was lowest, which was contrary to what has been reported by certain authors (Liu *et al.* 2009; Zhou *et al.* 2011; Haider *et al.* 2015). Haider *et al.* (2015) tested co-digestion of food waste and rice husks using S/I ratios of 0.25, 0.5, 1.0, 1.5, and 2.0 and obtained the highest TS and VS removals at an S/I ratio of 0.25 and the lowest removal value at an S/I ratio of 2.0. These researchers also found a strong relationship between the specific biogas yield and TS reduction (R^2 = 0.97) and VS (R^2 = 0.96). However, other authors have reported that there was no statistically significant correlation between the VS removal efficiency and the S/I ratio (Xu *et al.* 2012).

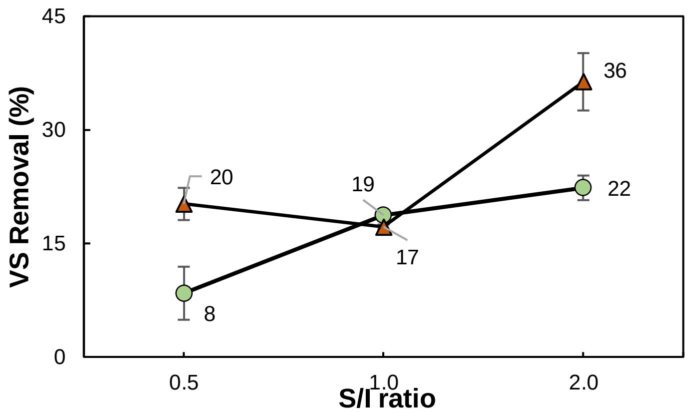

Fig. 3. Percent VS removal depending on the S/I ratio: Substrates correspond to TPW (●) and PPW (▲); error bars represent the standard deviation of each point.

Experiment B

The aim of this experiment was to evaluate the improvement in energy production compared with the results obtained in experiment A. Only TPW was used because this substrate yielded the highest methane production, lowest T80, and lowest lag phase values in experiment A.

Influence of the S/I ratio, temperature, and TS content on methane production

The highest yield occurred at an S/I ratio of 0.5 (Fig. 4). This result is consistent with that previously obtained in experiment A. The treatments in which the highest yields were obtained were 3B and 4B with 204.8 mL CH_4/g VS and 210.2 mL CH_4/g VS, respectively. There was no statistically significant difference between these two treatments. In contrast, the treatment with the lowest yield was 5B, in which an S/I ratio of 1.0 at 29 °C was used (Fig. 5).

Temperature had a positive effect on the methane production only for treatments where an S/I of 1.0 was used (p = 0.0001). Although the highest yield values were obtained at 39 °C at an S/I ratio of 0.5, the temperature had no significant effect on methane production at this S/I value. There were no statistically significant differences between the treatments at room temperature (Treatments 1A and 2A) and those at 29 °C with an inoculum concentration of 13.0 g VS/L (Treatments 1B and 5B). However, there were statistically significant differences between the treatments that were carried out at the higher end of mesophilic conditions (39 °C, Treatments 3B and 7B) with respect to those in which room temperature (22 ± 4.5 °C) was used at an inoculum concentration of 13.0 g VS/L (Treatments 1A and 2A). The temperature of 39 °C is within the optimal range of the mesophilic regime of digestion, 35 °C to 40 °C, so the highest yields of methane production were achieved using this temperature (Lettinga *et al.* 2001). Higher methane production was expected at a controlled temperature of 29 °C compared to an uncontrolled temperature; however, this could represent an advantage in conditions where it is not possible to establish a control of temperature.

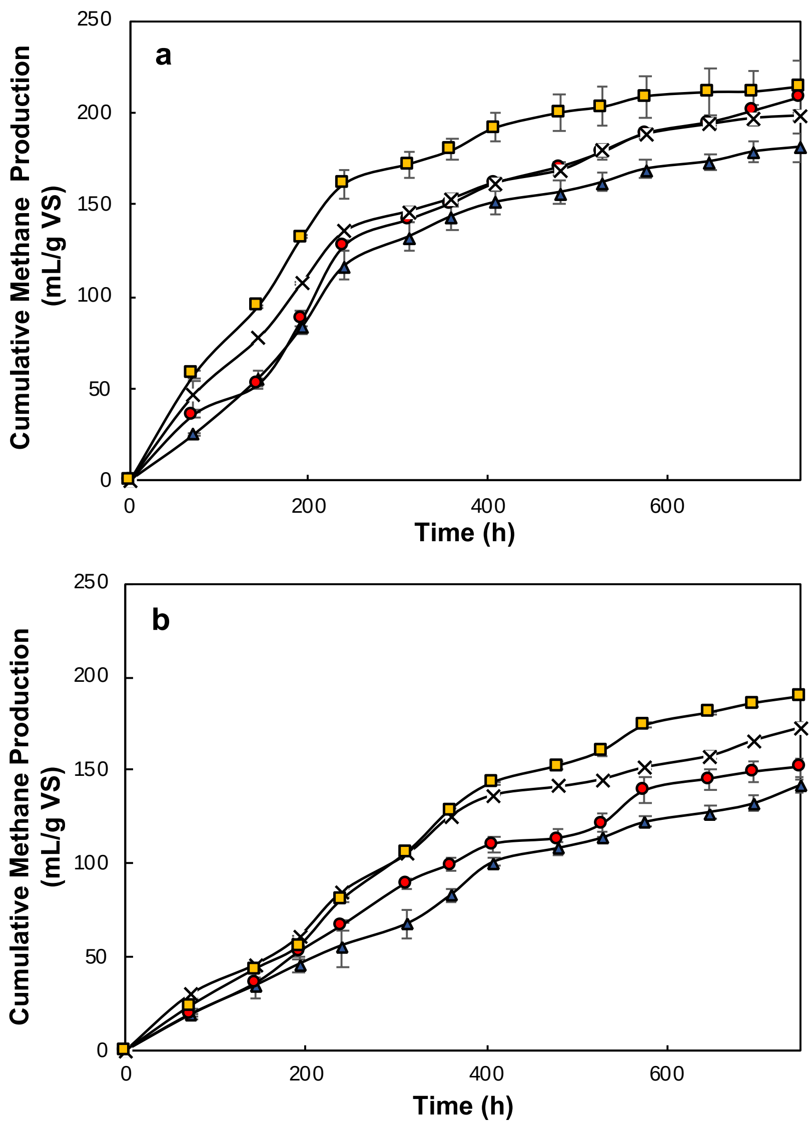

Fig. 4. Cumulative methane production from anaerobic digestion of TPW of experiment B at 0.5 (a) and 1.0 (b) S/I ratio; temperature and inoculum concentration correspond to 29 °C and 13.0 g VS/L (▲), 29 °C and 19.5 g VS/L (●), 39 °C and 13.0 g VS/L (x), 39 °C and 19.5 g VS/L (■); error bars represent the standard deviation of each point (triplicates).

The TS content had a positive effect on methane production at an S/I ratio of 0.5 ($p = 0.0195$) and at an S/I ratio of 1.0 ($p = 0.0045$). Thus, the increase in total solids percentage was reflected in higher methane production. These results are consistent with those reported by other authors that indicate an increase in biogas production as the total solids content in the mixture increases until it reaches an optimal production point (Budiyono *et al.* 2014; Yavini *et al.* 2014; Safari *et al.* 2018). Therefore, it is recommended to evaluate the optimal total solids content that generates the highest methane production using tomato plant residues considering that feedstocks had influence on TS content. In this study the percentages of TS in which the highest methane production was obtained at S/I of 0.5 and 1.0 (4.9 and 6.1%, respectively) are values far from the optimum points reported by other authors (Buyidono *et al.* 2010; Yavini *et al.* 2014).

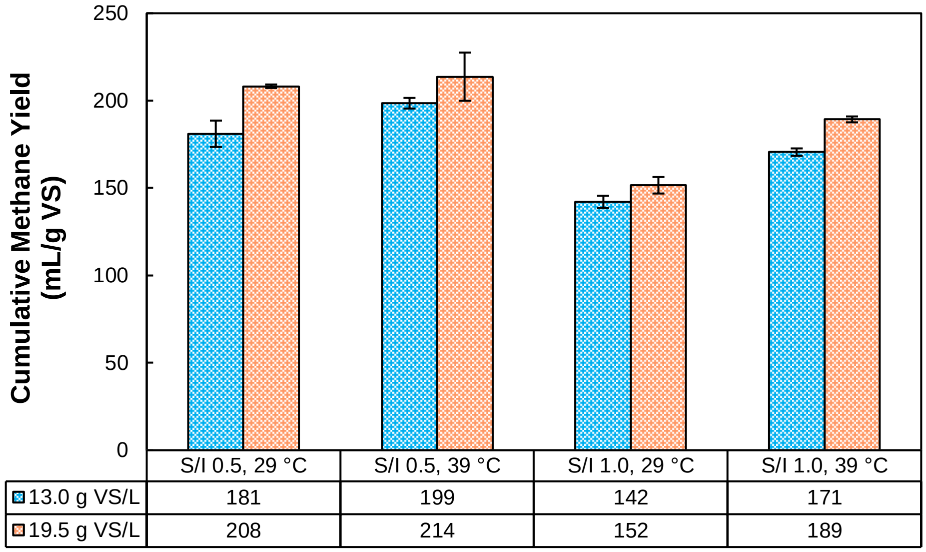

Fig. 5. Methane production yield for each of the treatments included in experiment B. The blue and orange bars indicate the treatments in which an inoculum concentration of 13.0 g VS/L and 19.5 g VS/L was used, respectively. Error bars represent the standard deviation of each point (triplicates).

Influence of the S/I ratio, temperature, and TS content on VS removal

In treatments 7B and 8B, similar VS removal percentages were obtained (41% and 42%, respectively), which were the highest VS removal values. In these treatments an S/I ratio of 1.0 was used. Based on the results from experiments A and B, the VS removal is greater at an S/I value of 1.0 than at 0.5.

In contrast to various studies reported that VS removal increases as the S/I ratio decreases (Liu *et al.* 2009; Zhou *et al.* 2011), different results have been obtained in other studies such as Córdoba *et al.* (2017). This study evaluated three S/I ratios (1.0, 3.0, and 6.0), and highest methane production was obtained from pig wastes at an S/I ratio of 1.0; however, the highest VS removal occurred at an S/I ratio of 3.0. The authors attributed this finding to the loss of volatile compounds during drying when performing the analysis to determine VS. Another reason for greater VS removals observed at high S/I ratios is due to the heterogenic sample affecting the results, as reported by Neves *et al.* (2004).

However, this study obtained similar results in both experiments, so it can be confirmed that higher substrate saturation (S/I ratio of 1.0) favored the cell synthesis of acidogenic bacteria. These bacteria use various metabolic pathways to generate other end metabolites instead of methane such as VFA and CO_2. Therefore, the production of these metabolites continues to contribute to VS removal (Li *et al.* 2016; Valdez- Vazquez *et al.* 2016).

Worth mentioning is that in the treatments in which an S/I ratio of 1.0 was used, there was a greater increase in pH at the end of the process compared with the treatments in which an S/I of 0.5 was used ($p < 0.05$) (Table 5).

Table 5. Initial and Final pH for Each Treatment of Experiment B for Methane Production from TPW

Treatment	Substrate	S/I	Temperature (°C)	% TS*	Initial pH	Final pH
1B	TPW	0.5	29.0 ± 1.0	3.2	6.90 ± 0.05	6.87 ± 0.01
2B	TPW	0.5	29.0 ± 1.0	4.9	7.13 ± 0.04	6.89 ± 0.03
3B	TPW	0.5	39.0 ± 1.0	3.2	6.73 ± 0.04	6.92 ± 0.07
4B	TPW	0.5	39.0 ± 1.0	4.9	7.00 ± 0.13	6.85 ± 0.06
5B	TPW	1.0	29.0 ± 1.0	4.1	6.69 ± 0.04	6.96 ± 0.02
6B	TPW	1.0	29.0 ± 1.0	6.1	6.81 ± 0.03	7.06 ± 0.07
7B	TPW	1.0	39.0 ± 1.0	4.1	6.68 ± 0.02	7.08 ± 0.03
8B	TPW	1.0	39.0 ± 1.0	6.1	7.01 ± 0.17	7.17 ± 0.06

*TS contributed by both the inoculum and the substrate

These results could be contradictory to what is expected, because a higher S/I ratio causes a greater accumulation of VFAs, and thus, system acidification could be reflected in a pH lower than neutral. However, the literature reports, even with a neutral pH, that the levels of certain individual acids may inhibit the process independently of the pH. For example, reportedly, the accumulation of propionic acid can occur at neutral pH, causing a decrease in the methane production yield (Yeole *et al.* 1996; Fang and Liu 2002). It is recommended to determine individual VFAs in further studies to confirm a greater accumulation of propionic acid at the S/I ratio of 1.0 using TPW. In contrast, Valdez-Vazquez *et al.* (2016) evaluated methane production from untreated wheat straw, reporting an increase in hydrolytic activity and VS removal when the nitrogen content and pH increased, although methanogenesis was inhibited (pH > 8.0). A similar phenomenon occurred in the current study, as there was an increase in pH in those treatments in which a greater VS removal was obtained.

Temperature had a significant effect on VS removal (p = 0.0064 and p = 0.0005 for 0.5 and 1.0 S/I ratios, respectively). At temperature of 39 °C a greater removal of VS was observed with respect to temperature of 29 °C (Fig. 6) as expected. A controlled temperature even though just 10° different in experiment B produced a greater removal of solids compared with the treatments performed at room temperature (experiment A).

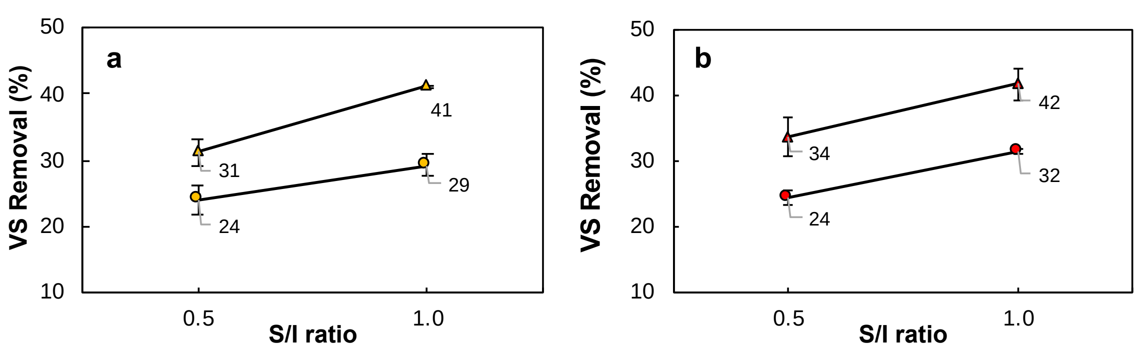

Fig. 6. Percent VS removal depending on the S/I ratio and temperature at inoculum concentration of 13.0 g VS/L (a) and inoculum concentration of 19.5 g VS/L (b): Temperature corresponds to 29 °C (●) and 39 °C (▲). Error bars represent the standard deviation of each point (triplicates).

At an S/I ratio of 0.5 at 39 °C (Treatment 3B) the VS removal was improved by 3.7 times the removal obtained at room temperature at the same S/I ratio (Treatment 1A).

Moreover, at an S/I ratio of 1.0 at 39 °C (Treatment 7B) improved VS removal of 2.2 times compared to the removal obtained at room temperature at the same S/I ratio (Treatment 2A).

The TS percentage had no significant effect on the %VS removal regardless of the S/I ratio used. Therefore, the volatile solids removal efficiency was not affected by the 50% increase in the TS content in the mixtures corresponding to treatments 1B, 3B, 5B, and 8B.

CONCLUSIONS

1. The type of substrate evaluated (TPW and PPW) had a significant effect on methane production and VS removal. At an S/I ratio of 0.5, a higher methane yield of 9.6% was obtained (mL CH_4/g VS) from TPW compared to PPW. In contrast to an S/I ratio of 1.0 this value was 6.9%. As for the VS removal, higher removal obtained using PPW compared to TPW.
2. The S/I ratio of 0.5 was most favorable for methane production, because the yield obtained was higher and the T80 values and the lag phase were the lowest with respect to the S/I ratio of 1.0. However, at this latter S/I ratio value the highest removal of VS was obtained. At an S/I ratio of 2.0 an inhibition of methanogenesis occurred.
3. There were no significant differences in methane production between treatments carried out at room temperature (22 ± 4.5 °C) with respect to those used at a controlled temperature of 29 °C with an inoculum concentration of 13.0 g VS/L. However, methane production showed an improvement of 17.3% and 23.6% when the temperature increased from room temperature to 39 °C in the treatments in which this same inoculum concentration was used at an S/I ratio of 0.5 and 1.0, respectively. The temperature had a significant positive effect on the VS removal, a higher removal was obtained at temperature of 39 °C.
4. A 50% increase in TS in the mixtures improved methane production, either in an S/I ratio of 0.5 or 1.0. In contrast, the TS content did not affect the VS removal.

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