# Silage of the organic fraction of municipal solid waste to improve methane production

Mario F. Castellón-Zelaya and Simón González-Martínez


## ABSTRACT

The silage of the organic fraction of municipal solid waste (OFMSW) is a common practice in biogas plants. During silage, fermentation processes take place, affecting the later methanisation stage. There are no studies about how OFMSW silage affects methane production. This work aimed to determine the effects of silage (anaerobic acid fermentation) at different solids concentrations and temperatures on methane production. OFMSW was ensiled at 20, 35, and 55 °C with total solids (TS) concentrations of 10, 20, and 28% for 15 days. The ensiled OFMSW was then tested for methane production at the substrate to inoculum ratios (S/I) of 0.5, 1.0, and 1.5. Independently of the temperature, the production of the metabolites during silage increases with decreasing solids concentration. The highest production was of lactic acid, ethanol, and acetic acid, representing together 95% of the total. Methane production from ensiled OFMSW at 10% solids concentration shows, under every tested condition, better methane production than from fresh OFMSW. Ensiled OFMSW produces more methane than fresh OFMSW, and methane production was highest at 35 °C.


**Key words** | anaerobic digestion, fermentation, methane production, OFMSW, silage

**Mario F. Castellón-Zelaya** (corresponding author)
**Simón González-Martínez**
Environmental Engineering Department,
Institute of Engineering,
National University of Mexico (Universidad Nacional Autónoma de México),
04510 Mexico City,
Mexico
E-mail: *mcastellonz@iingen.unam.mx*

## HIGHLIGHTS

- Ensiling OFMSW allows a better methane recovery than fresh OFMSW.
- Temperature and substrate-adapted inocula are essential for methanisation.
- Lower solids concentration allows higher acidification rates.
- Lactic acid and ethanol are the main products from OFMSW fermentation.
- Methanisation under mesophilic conditions is better from ensiled than from fresh OFMSW.



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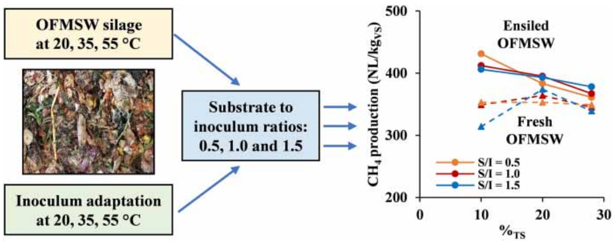
## GRAPHICAL ABSTRACT




## INTRODUCTION

Silage is a technique used since ancient times to preserve forages and other agricultural products. This technique has also been used as a biological pretreatment to maintain with minimal changes the natural moisture and nutrient content of agricultural residues and energy crops used to produce biogas (Bochmann & Montgomery 2013). As a stage before methanisation, silage implies that anaerobic digestion (AD) is carried out in two main stages (fermentation and methanogenesis), allowing the independent adjustment of the operating conditions. The whole AD microbial degradation process is divided into four consecutive biological processes: (1) the hydrolysis of complex organic molecules to soluble monomers takes place in the first step; (2) acidogenesis or fermentation is the process by which the soluble monomers from hydrolysis are converted to alcohols, volatile fatty acids (VFA), namely acetic, propionic and butyric acids, and $CO_2$ and hydrogen; (3) acetogenesis is the step where several of the previously produced VFA and alcohols are converted into acetate, which is an essential molecule used by methanogens as substrate; and (4) methanogenesis is the final step where different archaea can use acetate, $CO_2$ and hydrogen to produce methane as a final product. Better yields can be obtained when these processes are controlled separately (Schievano *et al.* 2014).

For homogeneous substrates such as maize, agricultural residues, and energy crops, it has been proven that silage before methanisation improves methane production (Herrmann *et al.* 2011). Ensiling conditions have a significant effect on the design and operation of reactors, as well as on the cost/benefit ratio of the process (Zupanĉiĉ & Grilc 2012). However, although the storage of the organic fraction of municipal solid waste (OFMSW) is a common practice in biogas plants, there are no studies about how silage of OFMSW affects methane production.

For the silage to be effective, rapid initial acidification is the key and the main operating factor is the solids concentration (Zhou *et al.* 2018), because the bioavailability of water affects microbial dynamics and reaction kinetics (García-Bernet *et al.* 2011). A commonly reported impact of moisture in silage is that higher total solids (TS) concentration slows down the bacterial growth and, consequently, leads to a limited fermentation, affecting substrate conservation. It has also been reported that low solids concentration is also associated with leachate formation during silage, producing nutrient and energy losses, causing severe pollution in receiving bodies and effluent management problems (Kung *et al.* 2018).

Another critical parameter for successful silage is temperature, given its effect on the enzymatic activity and thermal degradation of complex organic compounds (Buffière *et al.* 2018). At low temperatures, lactic fermentation may not be as efficient if there are not enough easily degradable substrates. If fermentation develops at lower temperatures, bacterial metabolism slows down to a point where hydrolysis cannot be achieved. It is essential that temperature does not become an obstacle for hydrolysis and that lactic acid can be produced as key for substrate conservation (Teixeira Franco *et al.* 2016).

There are few studies on how solids concentration and temperature during silage affect subsequent methane production. This has limited the understanding of how these two variables affect the biochemical processes of the anaerobic acid fermentation during silage and methane production from the digestates of this process (Teixeira Franco *et al.* 2016). Enough methanogenic microorganisms are needed to consume the metabolites produced during fermentation, thus avoiding acidification (Motte *et al.* 2013).

It is necessary to store OFMSW before processing it, and storage has been commonly made without any control during

days or weeks before processing it for methane production. Storage needs to be differentiated from silage: storage runs according to the production needs, and silage requires adjusting parameters to increase or decrease inherent processes. This research hypothesises that by adequately adjusting solids concentration and temperature during silage, better hydrolysis and, therefore, fermentation can be achieved. Higher VFA and alcohol concentrations are desired during fermentation because they represent better methane production during the subsequent methanisation. Therefore, this study's main objective was to determine the effects of OFMSW silage at different solids concentrations and temperatures on methane production. The relative concentration of methanogenic microorganisms, as substrate to inoculum ratio, S/I, was also evaluated.

## MATERIALS AND METHODS

### OFMSW sampling and characterisation

Source-separated OFMSW was collected at the Coyoacán solid waste transfer station in Mexico City, where sampling was made according to ASTM D5231-92 (2016). Approximately 200 kg from every one of 11 trucks was separated, and about 1 ton was thoroughly mixed using a skid-steer loader and shovels. Then, the quartering method was used two times to reduce the OFMSW to approximately 200 kg. Undesired materials, such as plastic bags, stones, and wood, were hand separated. Although the wastes were source-separated, different settlements delivered OFMSW with more or less undesired materials; these materials were discarded without quantification, and it can be positively concluded that these materials were less than 1% of OFMSW. The remaining 'clean' OFMSW was distributed in 2-L freezing bags and frozen at −20 °C.

OFMSW was characterised for pH, TS, volatile solids (VS), chemical oxygen demand (COD), VFA, and ammonia nitrogen ($NH_4$-N). Water-soluble carbohydrates (WSC) were also determined. The analytical determinations are described in the following sections.

### OFMSW silage

Eighteen batch 1,000-mL reactors with a reaction volume of 800 mL were operated at 20, 35, and 55 °C, for 15 days. Considering that 20 °C is the average annual temperature of Mexico City, this temperature was selected as silage/storage of OFMSW before processing under ambient temperature. Buffière *et al.* (2018) report that temperatures of 35 and 55 °C are optimal for microorganisms under mesophilic and thermophilic conditions, respectively.

For every temperature, OFMSW total solids concentrations were adjusted to 10, 20, and 28%, and duplicates were also prepared. Three reactors with different TS were connected to the biogas monitoring device (AMPTS II, Bioprocess Control, Sweden), and the other three were used for daily sampling. The three reactors connected to the biogas monitoring device were not opened, and their contents were not mixed during the 15 experimental days. The 28% solids concentration corresponds to the OFMSW as it was collected at the transfer station. Tap water was added to adjust the 10 and 20% solids concentrations.

The fermentation took place with the native microorganisms in OFMSW, and pH was not controlled. In the sampling reactors, the content was mixed manually before extracting samples. Daily, determinations were made for TS, VS, soluble COD, VFA, and alcohols. Volatile solids were corrected using the volatilisation factors proposed by Porter & Murray (2001): for 105 °C, the volatilisation factors were 0.394 for lactic acid, 0.937 for total VFA, and 1.0 for total alcohols.

### Methanisation of silage acid digestates

#### Adaptation of methanogenic inocula

Three differently adapted inocula were used for methanisation. Silage can be made at different temperatures (e.g., ambient temperature, 20 °C; several commercial dry-fermentation batch processes are mesophilic, 35 °C, and a few dry-fermentation batch processes are thermophilic, 55 °C). To follow the temperature concept, subsequent methanisation needed to be also at the same three temperatures. For this purpose, inocula containing methanogenic archaea was adapted from OFMSW at the same three temperatures. For the methanisation tests, these inocula were used fresh from the digesters where they were prepared.

For the inocula production (adaptation), OFMSW was mixed with anaerobic granular sludge from the wastewater treatment plant of a large brewery in Mexico City and allowed to digest, separately, under anaerobic conditions at 20, 35, and 55 °C. The three reactors were operated as semi-continuous, fed with fresh OFMSW. The following stability criteria were applied: (a) pH was maintained between 7.0 and 8.0 (Al Seadi *et al.* 2008), (b) bicarbonate alkalinity to total alkalinity ratio ($\alpha$ index) was higher than

0.6 (Martín-González *et al.* 2013), (c) VFA concentration was kept under 1.5 $g/kg_{VS}$ (Saveyn & Eder 2014) and (d) stable biogas production was considered when the methane concentration in the biogas was consistently over 60% (Schievano *et al.* 2008). Determination and control of alkalinities are necessary for efficient AD. Alpha ($\alpha$) index corresponds to the ratio of bicarbonate, or partial alkalinity (PA), to total alkalinity (TA) (Martín-González *et al.* 2013). Sun *et al.* (2015) consider $\alpha$-index under 0.5 during start-up as unstable but tolerable and 0.7 for regular operation. These digestates required weeks for adaptation before methanisation tests were performed. The methanisation tests were performed with fresh inocula from every reactor.

## Methanisation

The acid digestates obtained from the silage, described in the previous section, were subjected to methanisation to evaluate the fermentation efficiency. As a reference, fresh OFMSW also was methanised. To guarantee complete stability of the methanogenic digestates, before using them, for 6 days, they were kept under the same environmental conditions without feed. At the same silage temperatures (20, 35, and 55 °C), 18 reactors were operated in batch mode using 500-mL glass flasks. In every reactor, 10 $g_{VS}$ of methanogenic inoculum (see the previous section) was added, adjusting the S/I ratios to 0.5, 1.0 and 1.5, according to Table 1. Nine reactors were fed with acid digestates, and another nine reactors were fed with fresh OFMSW.

Immediately after filling the flasks, the air was washed out with nitrogen to guarantee anaerobic conditions. pH was not adjusted, and the experiment ran without buffer. All reactors were incubated without mixing for 25 days connected to an automated biogas counter (AMPTS II, Bioprocess Control, Sweden). The methane concentration in the biogas was determined daily with a gas chromatograph. A control containing only inoculum was also monitored to determine endogenous methane production.

**Table 1** | Adjusted parameters for methanisation

| | | Solids concentration during silage ($\%_{TS}$) | | |
|---|---|---|---|---|
| | **S/I ratio in methanisation** | **10** | **20** | **28** |
| Ensiled OFMSW | 0.5 | R1 | R2 | R3 |
| | 1.0 | R4 | R5 | R6 |
| | 1.5 | R7 | R8 | R9 |
| Fresh OFMSW | 0.5 | R10 | R11 | R12 |
| | 1.0 | R13 | R14 | R15 |
| | 1.5 | R16 | R17 | R18 |

In every reactor, 10 $g_{VS}$ inoculum was added.

## Sample preparation and analysis

Considering the heterogeneity of OFMSW, the samples were extruded using an Advance RH brand mill, model MOLAI - 22, with a sieve hole diameter of 8 mm. pH, total COD, total solids, volatile solids, and $NH_4$-N were determined according to Standard Methods (APHA 2017). Soluble COD was determined by photometry after 0.45 µm filtration. $NH_4$-N was determined in the liquid fraction after centrifugation at 5,000 rpm for 7 minutes. Lactic acid was determined using the spectrophotometric method proposed by Borshchevskaya *et al.* (2016). Ethanol and VFA (acetic, propionic, isobutyric, butyric, isovaleric, valeric and, hexanoic acids) were determined using a gas chromatograph (HP 5890 GC System) equipped with flame ionization detector (FID), Stabilwax column – DA, with hydrogen as carrier. The sample was previously filtered using 0.22 µm cellulose filters. Soluble carbohydrates were determined according to the phenol-sulphuric-acid method (Dubois *et al.* 1956).

The biogas composition ($CO_2$ and $CH_4$) was determined using a gas chromatograph (SRI 8610c) equipped with a thermal conductivity detector, stainless steel column packed with silica gel (8600-PK1A), helium as carrier gas with a flow of 27 mL/min. The detector temperature was 150 °C.

# RESULTS AND DISCUSSION

## OFMSW characteristics

Table 2 shows that OFMSW is a substrate with high moisture ($28\%_{TS}$) and, from the total solids, 80% corresponds to

**Table 2** | OFMSW characteristics.

| Parameter | Units | Value |
|---|---|---|
| pH | – | 5.2 ± 0.1 |
| Total solids | % | 28 ± 2 |
| VS/TS | – | 0.80 |
| Total COD | $g/kg_{VS}$ | 1,427 ± 132 |
| Soluble COD | $g/kg_{VS}$ | 540 ± 53 |
| WSC | $g/kg_{VS}$ | 275 ± 6 |
| Total VFA | $g_{COD}/kg_{VS}$ | 36 ± 3 |
| $NH_4$-N | $g/kg_{VS}$ | 1.4 ± 0.2 |

volatile solids. OFMSW is originally acid with a pH of 5.2 and VFA concentration of 36 $g_{COD}/kg_{VS}$. Determined as COD, 38% of OFMSW is soluble, and 51% of soluble COD corresponds to WSC. High WSC concentrations in OFMSW present advantages as the readily available carbohydrates during fermentation cause fast acid production and pH reduction. Lower WSC can cause a secondary fermentation during silage, and residual sugars and the previously produced lactic and acetic acids can be fermented to butyric acid, increasing pH (Pahlow *et al.* 2003).

## pH and total metabolites production during silage

Total metabolites are the sum of all products from the fermentation that takes place during silage. The typical metabolites from OFMSW fermentation are VFA, lactic acid, ethanol, and methanol (Saveyn & Eder 2014). These substances are all soluble and they were collected in the supernatant resulting from the centrifugation of the samples. COD was determined from the supernatant as an equivalent of total metabolites.

### Silage at 20 °C

Figure 1 shows that at 20 °C, the pH decreased steadily during the first 8 days and then increased slightly and again decreased to values near 4. Higher pH values were observed at higher solids concentrations. During the first 10 days, total metabolites (TM) production (as COD equivalent) increased to reach 220 $g_{COD}/kg_{VS}$, and only the metabolites from TS 10% continued to increase until day 15.

### Silage at 35 °C

pH decreased rapidly during the first 2 days, and then it remained stable near 4 until day 15. As with silage at 20 °C, the pH values increased with TS. TM from 20 and $28\%_{TS}$ increased rapidly during the first 3 days to reach values of approximately 220 $g_{COD}/kg_{VS}$, similarly to the silage at 20 °C. Only the TM for the $10\%_{TS}$ continued increasing until day 6, then it decreased to values near 300 and varied between 300 and 340 $g_{COD}/kg_{VS}$ until the end of the experiment.

### Silage at 55 °C

The silage at 55 °C behaved differently than the other two. pH decreased slowly from 5.5 to approximately 5 during the experimental time, and only the pH from $10\%_{TS}$ decreased faster than the other two TS cases. TM production for TS 20 and 28% was low compared to the other two temperatures with stable values between 20 and 30 $g_{COD}/kg_{VS}$. Like the other two temperatures, the lowest TS of 10% shows higher TM production.

In the three reactors with $10\%_{TS}$ (different temperatures), the drop in pH to values under 4.0 was caused by lactic acid production (Figure 2), whose pKa value is 3.86. At 55 °C (Figure 1), the decrease in pH was slighter than at lower temperatures, indicating a minimal fermentation because, at such high temperature, the growth of the native microflora in the substrate did not affect the process (McDonald *et al.* 1991).

Figure 1 shows that the lowest recorded pH values can be related to temperature and solids concentration. Lower pH is observed at lower temperatures and solids concentrations. Although TM are similar at the two lower temperatures, at 20 °C, pH is lower than at 35 °C. At lower solids concentrations, higher lactic acid productions can be observed (Figure 2). At lower temperatures, the solubility of the $CO_2$ produced during fermentation increases, contributing to decreasing pH. The same effect is reported by Weinberg *et al.* (2001), who ensiled maize and wheat at 37 and 41 °C and found that, at lower temperatures, $CO_2$

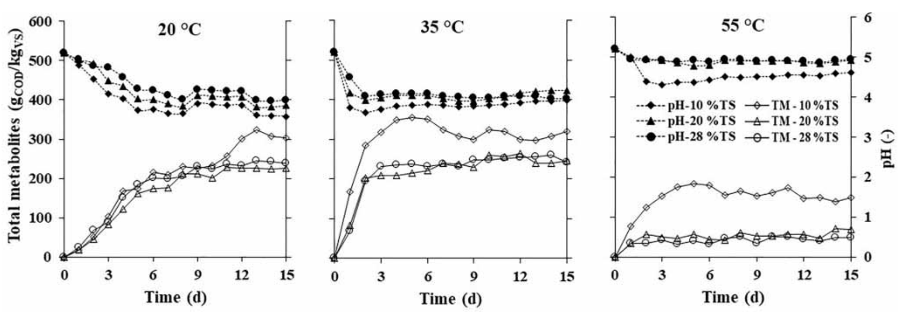



**Figure 1** | pH and total metabolites (TM) produced during OFMSW silage.

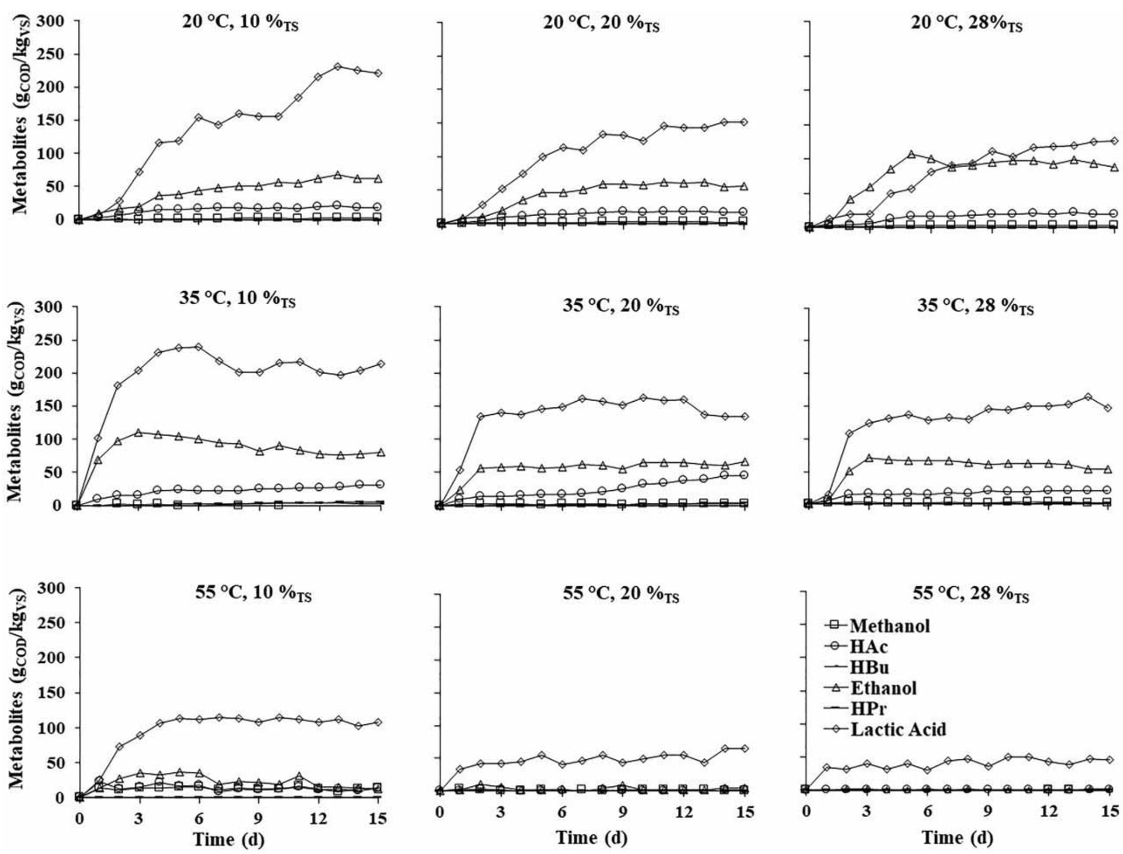



**Figure 2** | Metabolites production according to solids concentration and temperature.

decreased as the solubility increased, and pH resulted in lower values.

In terms of the effectiveness of the silage, a rapid decrease in pH is desirable, since at low pH values and if anaerobic conditions are maintained, the growth of all microorganisms, including lactic acid bacteria (LAB), is inhibited, allowing the conservation of the substrates (McAllister & Hristov 2000). This research concludes that the rate with which pH decreased is strongly influenced by temperature. Although the final pH values do not show a significant difference between 20 and 35 °C, at 35 °C, pH decreased more rapidly than at the other two temperatures. While at 35 °C, pH reached a minimum value and stabilised at day 3 of silage, at 20 °C, it took 13 days for the ensiled material to reach stable values.

The highest TM production, including all cases shown in Figure 1, was recorded with the lowest TS; lower TS concentrations allow water to function as a diluting agent. The concentration of the metabolites decreases as TS decreases, increasing microbial activity. Using a different argument, García-Bernet *et al.* (2011) report that, at higher solids concentration, substrate bioavailability is low, negatively affecting the microbial activity: mass transfer is limited, and the microorganisms have difficulty accessing the soluble fraction of the substrate. Considering the final concentrations of total metabolites, there is no significant difference between 20 and 35 °C. The effect of the temperature on the rates at which metabolites are produced is noted. At 35 °C, the primary TM production took place during the first 3 days of silage, while at 20 °C, it took 13 days for the ensiled material to reach stable TM concentrations.

## Different VFA, lactic acid, and alcohols production during silage

The metabolites with higher concentrations detected during silage were lactic acid, ethanol, and acetic acid, representing more than 95% of the total metabolites (Figure 1). Methanol, propionic and butyric acids were also detected. As lactic acid is the primary metabolite detected at all tested temperatures, it indicates the dominance of LAB in the microbial community as native flora in OFMSW. Higher ethanol and

acetic acid concentrations suggest that, during silage, the primary fermentation pathway was heterolactic. Vervaeren *et al.* (2010) report that ensiling for heterolactic fermentation is convenient for subsequent methanisation because both ethanol and acetic acid can be readily used for methane production. McDonald *et al.* (1991) conclude that heterolactic fermentation increases the loss of organic material through $CO_2$ production and that heterolactic fermentation increased methane production; nevertheless, increasing methane production did not compensate for the overall materials and energy losses.

### VFA, lactic acid, and alcohols production at 20 °C

Figure 2 shows that lactic acid is the primary metabolite produced under all tested conditions. The highest lactic acid production was recorded for the lowest solids concentration ($10\%_{TS}$), independently of the temperature, with maximum concentrations of 221, 151, and 127 $g_{COD}/kg_{VS}$, for 10, 20, and $28\%_{TS}$, respectively. Ethanol constituted the second-highest concentration of metabolites produced under every one of the three tested temperatures. The highest ethanol production was recorded on day 7 at $28\%_{TS}$ with 100 $g_{COD}/kg_{VS}$, and it remained constant until day 15. For 10 and $20\%_{TS}$, the highest concentrations were reached at day 12 and 8, respectively, with values of 60 $g_{COD}/kg_{VS}$. Two pathways can lead to ethanol production: (1) heterolactic fermentation, where, besides lactic acid, ethanol and acetic acid are also produced, and (2) alcoholic fermentation. Muck (2010) reports that ethanol can be produced through the fermentation of OFMSW and other substrates. Even at lower pH values, as long as enough WSC are present, yeast can develop and produce ethanol from ensiled substances.

From the three VFA tested, only acetic acid was detected at the three evaluated solids concentrations, and no significant difference was observed among them. Concentrations of 19, 18, and 21 $g_{COD}/kg_{VS}$ were observed for the reactors with 10, 20, and $28\%_{TS}$, respectively. These concentrations represent between 6 and 8% of TM produced, higher than the values between 1 and 3% reported by Kung *et al.* (2018).

### VFA, lactic acid, and alcohols production at 35 °C

Similar to 20 °C, the highest lactic acid production at 35 °C was recorded at the lowest solids concentration (214, 134, and 147 $g_{COD}/kg_{VS}$, for 10, 20, and $28\%_{TS}$, respectively). The lactic acid concentration at 10% reached a maximum of 240 $g_{COD}/kg_{VS}$ on day 6. After this day, the concentration decreased, caused by butyric fermentation. This fermentation pathway is common for the genus *Clostridium tyrobutyricum*, where two moles of lactic acid are necessary to produce 1 mole of butyric acid: 2 lactic acid $\rightarrow$ 1 butyric acid $+2\ H_2 + 2\ CO_2$ (Pahlow *et al.* 2003).

Slightly higher lactic acid production rates were observed at 35 °C than at 20 °C. The main difference from 20 °C is that at 35 °C, the highest concentrations were achieved after only 6 days for $10\%_{TS}$, 3 days for 20 and $28\%_{TS}$. The effect of temperature could be observed mainly on the production rates and not on the predominance of lactic fermentation during ensiling. Although higher lactic acid production is convenient for methane production given its viability as an intermediate metabolite in anaerobic digestion, the methanisation of a substrate previously subjected to lactic fermentation can be inhibited by low pH (Zhou *et al.* 2018).

Differently from the results at 20 °C, the highest ethanol production at 35 °C was recorded at day 4 with 105 $g_{COD}/kg_{VS}$ under the lowest solids concentration of $10\%_{TS}$. Considering that higher acid concentrations limit the activity of yeasts, it can be concluded that ethanol production is associated with heterolactic fermentation. In this fermentation pathway, one mole of ethanol is produced from every mole of lactic acid. This is coherent with Figure 2, as the curve for ethanol under 28% decreases when lactic acid increases. According to Zhao *et al.* (2016), during methanisation, ethanol has a high potential to improve the alkalinity of the system and, therefore, its buffering capacity, since a significant fraction of substrate carbon is converted into ethanol and not into VFA. Wu *et al.* (2015) state that thermodynamically, a fermentation oriented to ethanol production allows methanogenic microorganisms a better energy recovery.

At 35 °C, low concentrations of acetic acid were detected. The highest acetic acid concentration was 50 $g_{COD}/kg_{VS}$ at $20\%_{TS}$, while at 10 and $28\%_{TS}$, maximum concentrations were 33 and 22 $g_{COD}/kg_{VS}$, respectively. Variations in acetic acid production can be associated with acetogenesis from ethanol and lactic acid. Figure 2 shows that, as acetic acid increased, ethanol and lactic acid decreased.

From day 6, propionic acid was detected at 10 and $20\%_{TS}$, increasing its concentration to 5 $g_{COD}/kg_{VS}$ on day 15. Butyric acid was detected only at $10\%_{TS}$, with a maximum of 4.0 $g_{COD}/kg_{VS}$.

### VFA, lactic acid, and alcohols production at 55 °C

At 55 °C, lactic acid production was considerably lower than at 20 and 35 °C, because, at temperatures higher than 50 °C,

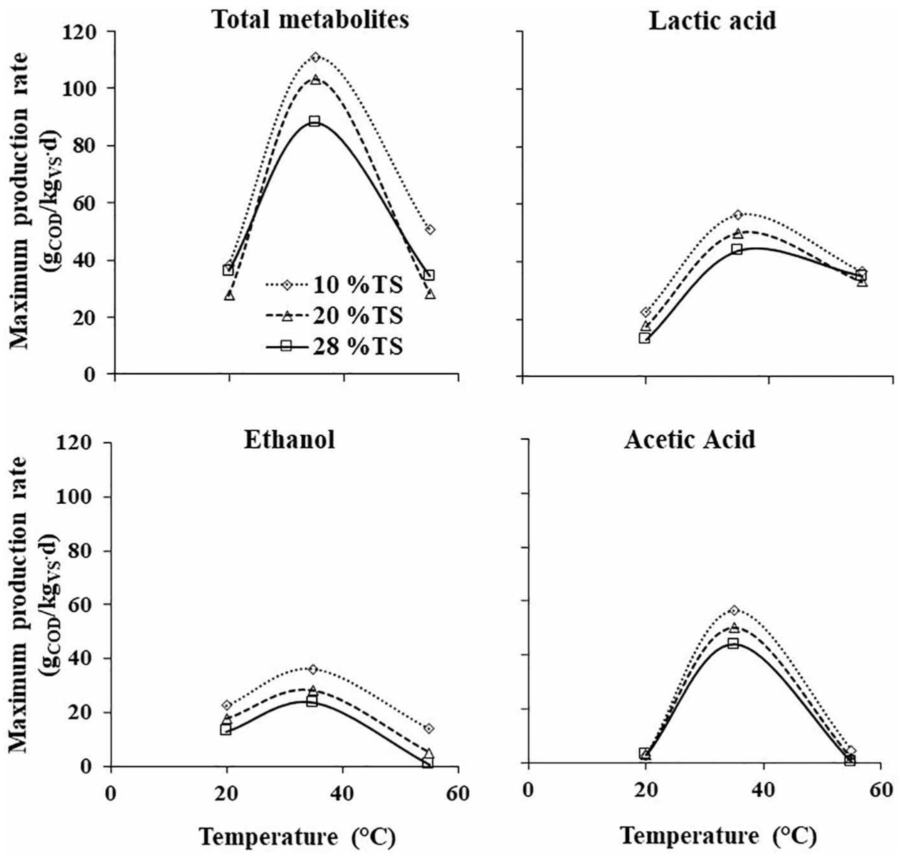



**Figure 3** | Maximum production rates of ethanol and acetic and lactic acids.

the growth of LAB is strongly restricted: the optimal temperature range for the development of LAB varies between 27 and 38 °C (McDonald *et al.* 1991). Acetic acid was the only VFA detected. Lower acetic acid and ethanol concentrations were recorded only in the silage at $10\%_{TS}$. For both acetic acid and ethanol, the maximum concentration was approximately 50% lower than at 20 and 35 °C. These results agree with the conclusions of McDonald *et al.* (1991) because LAB and alcohol-producing yeasts are inhibited at higher temperatures.

## Fermentation rates

The maximum TM production rates of the three primary metabolites were evaluated as a function of solids concentration and temperature on fermentation during ensiling. From Figure 2, the maximum initial rates for acetic and lactic acids, and ethanol, were calculated and plotted in Figure 3. Most of the highest rates were detected at the beginning of every reaction. Figure 3 shows slight rate differences among the solids concentration values, but the temperature is an important parameter.

TM present similar rates at 20 and 55 °C, and, at 35 °C, the rates increase significantly to values between 85 and 130 $g_{COD}/kg_{VS}\cdot d$, where clearly, the rates behave inversely with TS. For **lactic acid**, the rates are lowest with values near 20 $g_{COD}/kg_{VS}\cdot d$ at 20 °C and increase three-fold at 35 °C; at 55 °C the rate values decrease to approximately 35 $g_{COD}/kg_{VS}\cdot d$. The production rates of **ethanol** are low at 20 °C, increase from 20 to 35 $g_{COD}/kg_{VS}\cdot d$ at 35 °C and are negligible at 55 °C. The **acetic acid** production rates at 20 and 55 °C are almost zero, but, at 35 °C, the production is high with values between 42 and 55 $g_{COD}/kg_{VS}\cdot d$, indicating a clear preference of the microorganisms for 35 °C.

## Characteristics of the adapted digestate (inoculum)

The procedure to produce the adapted digestates that were used for methanisation is explained in Materials and Methods. The main characteristics of the digestates used as inoculum for methanisation are presented in Table 3.

**Table 3** | Characteristics of the produced digestates at three different temperatures

| Parameter | Units | 20 °C | 35 °C | 55 °C |
|---|---|---|---|---|
| pH | – | 7.7 | 8.4 | 8.0 |
| Total solids | g/kg | 120 | 104 | 26 |
| Volatile solids | g/kg | 89 | 75 | 18 |
| Alpha index | – | 0.61 | 0.78 | 0.71 |
| $CH_4$ in biogas | % | 69 | 70 | 71 |
| Total VFA | $g_{COD}/kg_{VS}$ | 2.0 | 1.3 | 1.6 |

## Methanisation of digestates from the acid fermentation

For methanisation, the digestates from the acid fermentation were placed in flasks in water baths at 20, 35, and 55 °C together with the previously adapted inoculum. The detailed conditions are presented in Table 1. For these tests, pH was determined immediately after the substrate and inoculum were placed in the flasks (initial) and at the end (final) of the 25 days of methanisation (Supplementary Table 1). Initial pH values were inversely proportional to the S/I ratio and directly proportional to temperature. Final pH values did not show significant differences between the temperatures of 35 and 55 °C. At lower temperatures, the ensiled OFMSW were acidic and, as the S/I ratio increased, the adapted inoculum was not able to neutralise the pH. For the experiments at 35 and 55 °C, pH increased during methanisation and finalised with values between 7.8 and 8.2, with only one exception finished with 7.3. According to Al Seadi *et al.* (2008), adequate pH values for methanisation need to be between 7 and 8.

Considering that different substrates, under the influence of different ambient conditions, are consumed at different rates, time differences need to be expected. To calculate the corresponding value for methane production and reaction time, the recommendation of Holliger *et al.* (2016) was used; they consider that the final methane production, and the corresponding reaction time, are reached when the accumulated methane values do not change more than 1% as an average of three consecutive days when the methane production can be monitored in continuous systems with differential measuring devices. The reaction time is the time required for the microorganisms to metabolise exogenous substrates. This is reflected directly as methane production, and it can be obtained from the methane production curves in Figure 4.

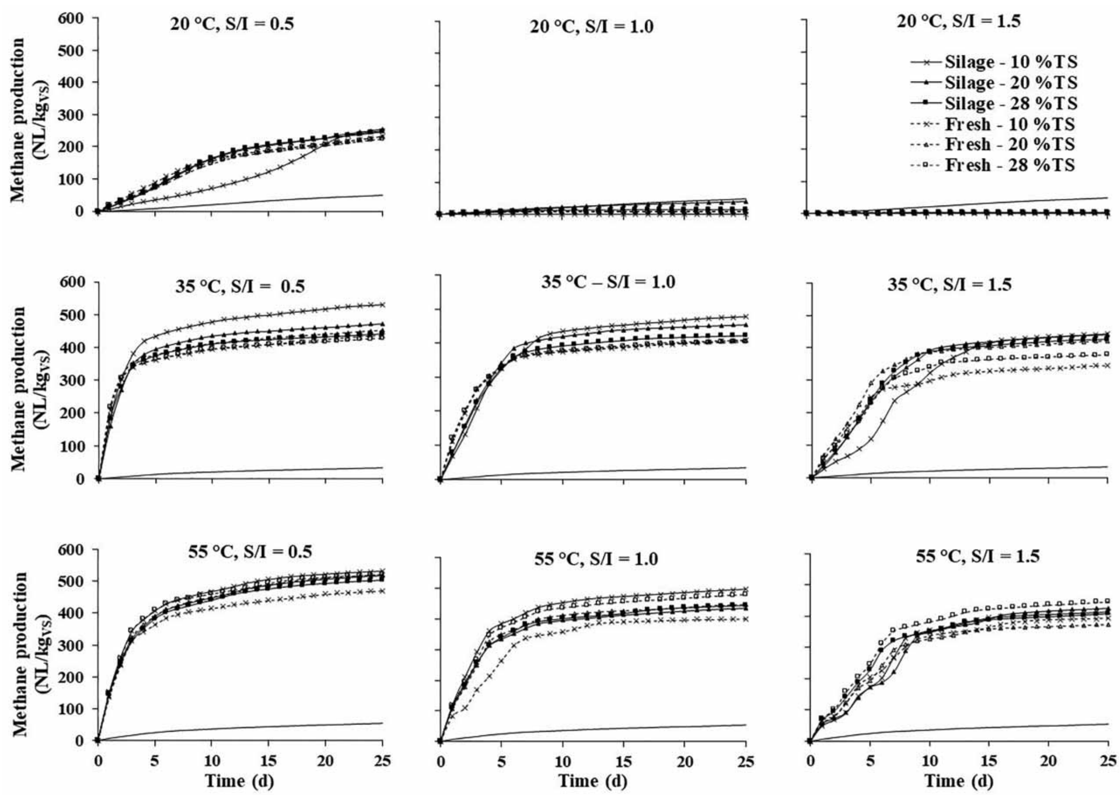



**Figure 4** | Methane production of ensiled and fresh OFMSW. The continuous line represents the blank containing only inoculum.

## Methanisation at 20 °C

Except for the cases with the lowest S/I ratios, the initial pH values for ensiled OFMSW were acidic (Supplementary Table 1), and these values did not change during the 25 days, making the methane production deliver lower values than the blank (Figure 4). Only for S/I = 0.5, the pH was slightly alkaline, and methane production could be observed. The final values for methane production are in Table 4. Except for fresh OFMSW, the other five cases showed similar behaviour with similar final values. Under psychrophilic conditions, microbial metabolism is slower than at mesophilic or thermophilic ones. Rajagopal *et al.* (2017) report relatively high methane production from agro-food, industrial, and livestock wastes at 20 °C; they show methane production from 401 to 457 NL/kg, but, unfortunately, they do not report any relationship with pH values. Without mentioning the influence of pH, Muñoz (2019) reports much lower values than Rajagopal *et al.* (2017) with 59.3 to 116.5 $NL/kg_{VS}$.

From the nine cases run at 20 °C, only methane production for S/I = 0.5 is reported. For higher S/I ratios, the methane production resulted in values under the blank. As the pH values for S/I 1.0 and 1.5 were all acidic, methane production was inhibited, and practically no production could be recorded. According to Table 4, methane production from ensiled OFMSW was 16% higher than for fresh OFMSW. The reaction times were 22 days for ensiled OFMSW and 16 days for fresh OFMSW.

## Methanisation at 35 °C

At 35 °C, the initial pH was recorded between 6.0 and 7.6 for both ensiled and fresh OFMSW. Fresh OFMSW initiated

**Table 4** | Methane production and reaction times after subtracting the blank (inoculum without substrate)

| | TS in silage (%) Ensiled OFMSW | S/I ratio for methanisation Fresh OFMSW | $CH_4$ production ($NL/kg_{VS}$) | | $\Delta CH_4$ production (%) | Reaction time (d) | |
|---|---|---|---|---|---|---|---|
| | | | Ensiled OFMSW | Fresh OFMSW | | Ensiled OFMSW | Fresh OFMSW |
| **20 °C** | **10** | **0.5** | 167 | 144 | + 16 | 22 | 16 |
| | | **1.0** | ND | ND | ND | ND | ND |
| | | **1.5** | ND | ND | ND | ND | ND |
| | **20** | **0.5** | 164 | 144 | + 14 | 16 | 16 |
| | | **1.0** | ND | ND | ND | ND | ND |
| | | **1.5** | ND | ND | ND | ND | ND |
| | **28** | **0.5** | 156 | 137 | + 14 | 16 | 16 |
| | | **1.0** | ND | ND | ND | ND | ND |
| | | **1.5** | ND | ND | ND | ND | ND |
| **35 °C** | **10** | **0.5** | 431 | 349 | + 23 | 11 | 6 |
| | | **1.0** | 412 | 353 | + 17 | 11 | 9 |
| | | **1.5** | 406 | 314 | + 29 | 17 | 14 |
| | **20** | **0.5** | 383 | 364 | + 5 | 10 | 10 |
| | | **1.0** | 395 | 353 | + 12 | 10 | 9 |
| | | **1.5** | 393 | 374 | + 5 | 13 | 11 |
| | **28** | **0.5** | 361 | 345 | + 5 | 10 | 10 |
| | | **1.0** | 367 | 349 | + 5 | 10 | 9 |
| | | **1.5** | 378 | 339 | + 12 | 12 | 12 |
| **55 °C** | **10** | **0.5** | 372 | 329 | + 13 | 8 | 8 |
| | | **1.0** | 407 | 340 | + 20 | 10 | 14 |
| | | **1.5** | 366 | 320 | + 14 | 16 | 12 |
| | **20** | **0.5** | 350 | 354 | − 1 | 8 | 8 |
| | | **1.0** | 348 | 364 | − 4 | 9 | 10 |
| | | **1.5** | 377 | 327 | + 15 | 18 | 15 |
| | **28** | **0.5** | 343 | 368 | − 7 | 8 | 8 |
| | | **1.0** | 354 | 390 | − 9 | 9 | 10 |
| | | **1.5** | 354 | 390 | − 9 | 15 | 15 |

$\Delta CH_4$ represents the variation in $CH_4$ production of ensiled OFMSW with respect to fresh OFMSW.
ND, not detected. Methane production from the substrate was lower than from the inoculum.

with slightly higher pH values than ensiled OFMSW. Initial pH was higher for lower S/I ratios. Without exception, pH increased during the 25 days of methanisation to finish with values between 7.3 and 8.2, indicating that no VFA accumulation took place and that methanisation ran without incidents (Supplementary Table 1).

The methane production curves in Figure 4 show: (1) lower silage concentrations produce more methane; (2) as the S/I ratios increase, the methane production decreases; (3) the initial methane production rates decrease with increasing S/I ratios; (4) reaction times increase with S/I ratio (compare with Table 4).

Methane production (Table 4) from ensiled OFMSW is 5 to 29% higher than from fresh OFMSW, reporting the highest value for ensiled OFMSW at $10\%_{TS}$ with 431 $NL/kg_{VS}$. Schievano *et al.* (2014) reported an increase between 23 and 43% when comparing methane production from ensiled and not-ensiled corn silage and fruit and vegetable wastes in a two-stage process. Liu *et al.* (2006) observed a 21% increase in methane production from the digestion of domestic solid waste in batch reactors operated in two stages. On the other hand, Voelklein *et al.* (2016) recorded a 23% increase in methane production when they used food as a substrate for the two-stage anaerobic digestion. For the anaerobic co-digestion of cattle manure and corn silage in two-stage systems, Buffière *et al.* (2018) state that mesophilic methanisation is more effective than psychrophilic or thermophilic because the microorganisms adapt better to this temperature and achieve better hydrolysis than at other temperatures. They found that that silage improved the accessibility of the particles without necessarily causing their solubilisation. As mesophilic AD of solid wastes is more stable and versatile than at other temperatures, most installations producing methane operate under these conditions, and the microorganisms can tolerate temperature variations of $\pm 3\,^{\circ}C$ without affecting the process (Zupanĉiĉ & Grilc 2012).

### Methanisation at 55 °C

Initial pH values for ensiled OFMSW were slightly alkaline, with values between 6.9 and 7.5. For fresh OFMSW, the initial pH values fluctuated between 7.3 and 7.7. After the 25 days of methanisation, pH values were between 7.8 and 8.1 for ensiled OFMSW and between 8.0 and 8.2 for fresh OFMSW. VFA did not accumulate and no pH inhibition needed to be taken into account.

The methane production curves in Figure 4 present a slightly different behaviour for the other two temperatures: after the rapid methane production, a second step with a much lower production rate can be observed. The methane production values were extracted after this second step, which also required longer reaction times. Similar to the cases of methanisation at 20 and 35 °C, the methane production, reaction times, and production rates decreased with increasing S/I ratios.

Table 4 shows that methane production from the digestates at 10% was higher for ensiled than for fresh OFMSW. For digestates at 20%, methane production of ensiled and fresh OFMSW was similar, and at 28% solids, the methane production of fresh OFMSW was higher than for ensiled OFMSW.

Advantages and disadvantages can be drawn from methanisation at 55 °C. The benefits are: (1) methane production can only be recommended for lower solids concentrations; (2) the S/I ratio does not affect methane production significantly; (3) reaction times are shorter at 35 °C. The disadvantages are: (1) between 9 and 15% lower methane productions can be achieved when compared with mesophilic methanisation; (2) at higher solids concentrations, fresh OFMSW produces more methane than the ensiled one.

The highest methane production was between 366 and 402 $NL/kg_{VS}$, for 10% solids. These values are still higher than those reported by Fernández-Rodríguez *et al.* (2014), who obtained methane between 190 and 340 $NL/kg_{VS}$. These last authors also report that methane production decreases with increasing solids concentration, concurring with the results of this research. Fdéz-Güelfo *et al.* (2010) report much lower values than the ones from the previously mentioned authors, concluding also that methane production decreases with increasing solids concentration.

## CONCLUSIONS

This work deals with silage (fermentation) of OFMSW under three different temperatures and solids concentrations. The processes were analysed, and the products characterised. The products were subject to methanisation to determine the effects of OFMSW silage on methane production. The most important conclusions are as follows:

- Independently of the temperature, the production of the metabolites increases with decreasing solids concentration. The main metabolites produced were lactic acid, ethanol, and acetic acid, representing together 95% of the total. Lactic acid is the primary metabolite produced at every one of the three tested temperatures.
- The highest metabolite production during silage was achieved at 35 °C and the lowest solids concentration of 10%.

- At 35 and 55 °C, during methanisation, the pH values were slightly alkaline, indicating that no acids accumulated and that the adapted inocula performed adequately.
- At 20 °C, the methanisation of ensiled and fresh OFMSW, at high S/I ratios, was inhibited by acid accumulation.
- Methane production from ensiled OFMSW at $10\%_{TS}$ shows, under every tested condition, better methane production than from fresh OFMSW.
- Ensiled OFMSW produces more methane than fresh OFMSW, and the production increases with decreasing substrate to inoculum ratio. Methane production was highest at 35 °C.
- For methanisation of ensiled and fresh OFMSW, solids concentration is the controlling parameter, and the substrate to inoculum ratio is the controlling parameter for reaction time.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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