Methane production after fermentation of the organic fraction of municipal solid waste for the selective production of metabolites under different pH and reaction times

G. Dimitriv Jojoa-Unigarro*(ORCID: 0000-0001-9256-6850),
Simón González-Martínez (ORCID: 0000-0002-0452-195X)

Institute of Engineering, National University of Mexico
(Universidad Nacional Autónoma de México). Mexico City, Mexico
*gjojoau@iingen.unam.mx

Abstract

Thermodynamically, lactic acid and ethanol allow the best energy recovery among all common metabolites for methane production. When faster methane production is required, previously fermented organic fraction of the municipal solid waste (OFMSW) can be essential for methane production. This research dealt with the fermentation of OFMSW under pH values of 4, 5, and 6, at 35°C, in batch reactors. Samples were taken at reaction times of 1, 3, and 6 days for every pH. Samples were characterized and exposed to methane production at 35°C using an automatic biogas measurement device. Lower pH and shorter fermentation allow better ethanol and lactic acid production. Without exception, the liquid digestates produce more methane than the solid ones. Fermented OFMSW produced 19% more methane than the unfermented one. The first methanization stage of the liquid fractions requires 1 to 2 days, and the solid fractions from 4 to 8 days. Both fractions together represent 49 to 69% of total methane production. The Michaelis & Menten Vmax values are significantly higher for the liquid fractions than for the solid ones. Vmax substantially increases with decreasing pH. The proportionality constant Km for liquid digestates is significantly lower than for the solid ones, indicating a good affinity of the methanogens for the substrate. Independently on pH and duration, fermentation allows faster methanization.

Keywords. *Alcoholic fermentation; lactic fermentation; anaerobic digestion; methane production*

INTRODUCTION

Anaerobic digestion (AD) is a well-known technology for producing methane from different substrates. The AD microbial 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 [1, 2, 3, 4].

pH and reaction time affect the metabolic pathways during the organic fraction of municipal solid waste (OFMSW) fermentation. Recent studies point out that acid fermentations lead to ethanol production and that the subsequent methanization runs faster because of a more efficient electron transport among methanogens [5, 6, 7, 8, 9, 10]. The specialized literature often finds how one parameter affects ethanol or acetic acid production. Still, literature on how both parameters affect ethanol and acetic and lactic acid production simultaneously cannot be easily found. For OFMSW fermentation, Wu et al. [5] and Zheng et al. [11] indicate that the optimal pH for ethanol fermentation lies between 4 and 5 and lactic acid production between 5 and 5.5. Cheah et al. [12], Dahiya et al. [13], and Jankowska et al. [14] observed that neutral or slightly alkaline pH values produce mainly volatile fatty acids (VFA) and that pH values between 4 and 5 inhibit VFA production.

Wu et al. [5] worked with food wastes, and they evaluated the selectivity during fermentation in a semicontinuous reactor under pH values from 5 to 7. They found that, at pH 5, the selectivity was mainly for acetic acid with traces of propionic and butyric acids. At pH 6, butyric acid increased to 60% of the total metabolites; the rest was acetic. At pH 7, acetic and butyric acids predominate, and propionic acid represents only 15%. Unfortunately, they did not analyze the presence of ethanol or lactic acid. Wu et al. [15] fermented food wastes at pH 6 for 24 days in a batch reactor: ethanol appeared on the first day and disappeared on the fifth day. When the butyric acid concentration decreased, acetic acid increased to 40-45%. The rest was propionic and valeric acids.

Reaction time affects fermentation because the available substrates (mono- and disaccharides) for ethanol production can be rapidly depleted, and, after, the ethanol can be transformed into other undesired products. Acid fermentation (VFA and lactic) requires longer times because the microorganism can use more complex substances as substrate and thus longer reaction times. For fermentation times above three days, microorganisms can transform ethanol and lactic acid into acetic acid through acetogenesis [12, 16].

Anaerobic digestion has proven to be thermodynamically more effective when the intermediaries before methanization are ethanol and lactic acid [5, 17, 18]. Using pure substrates for methanization, Jojoa-Unigarro and González-Martínez [19] proved that methane production from acetic acid has the highest yield and that methane from ethanol report the highest reaction rates; it is unclear if acetogenesis of ethanol is faster than methanogenesis of acetic acid or simply because methanogens use ethanol directly for methane production. They also demonstrated that acetogenesis of propionic, butyric, and lactic acids is the limiting step for methane production.

Most studies about fermentation, as a previous step of methanization, focus on one single intermediary as a research objective without quantifying the other metabolites produced as side reactions [6, 8, 12, 20, 21]. They also observe that higher methane yields can be obtained by separating the fermentation from methanization; they also point out that lower organic loading rates are required to avoid the accumulation of metabolites that can inhibit methanization. To prevent inhibition caused by substrate accumulation, some authors recommend separating the water-soluble fraction from the solid one (particulate) in OFMSW and using only the liquid fraction for methane production, discarding the solid one. The argument is that the solid substances are mainly lignocellulosic that do not contribute to methane production [22, 23]. By doing this, the reaction time for methanization can be dramatically reduced. According to Campuzano and González-Martínez [24], this procedure is not as good because the solids extracted from OFMSW contain not water-soluble substances and, when exposed to methanogens, can produce large quantities of methane. The disadvantage is that they require more significant reaction times, but they can produce methane.

The substances in the liquid can be readily transformed into methane, and the microorganisms can "concentrate" in the solid fraction. Like the researches of Bo et al. [20], Komemoto et al. [21], Cheah et al. [12], Zhao et al. [6], and Zou et al. [8], working with fruits and vegetable wastes, Bacab et al. [25], Fezzani and Cheikh [26], Li et al. [27], and Majhi and Jash [28], separating liquids from solids after fermentation, conclude that the substances contained in the liquid fraction can be methanized in shorter times. When the waste is subjected to one-step anaerobic digestion, the process requires approximately 21 days; if the process is separated into fermentation and methanization, the required reaction times are 3 to 5 days for fermentation plus 5 to 9 days for methanization.

No literature has been found on ethanol production from OFMSW, considering lactic acid as an essential byproduct and its effects on acetogenesis and VFA production. The main objective of this research was to analyze the selectivity and production of metabolites during the fermentation of OFMSW under different, controlled pH and reaction times. A second objective was to determine how the diversity of the fermentation products in the soluble and particulate fractions affect methanization. The Michaelis & Menten model was used for the kinetic analysis.

MATERIALS AND METHODS

Fermentation Reactor with Automatic pH Control

A 4-liter reactor with an active volume of 3.0 L was operated at 35°C. The reactor has a twin-bladed mechanical mixing (Bioprocess Control, Sweden) and automatic pH control consisting of an electrode, a control device, and two peristaltic pumps for solutions of NaOH 2 M and H_3PO_4 2 M (Figure 1). For every batch, pH was adjusted automatically at 4.0, 5.0, and 6.0, depending on the experimental stage. According to the recommendations of Wu et al. [15], to avoid inhibition caused by substrate, the volatile solids concentration was maintained at 4%.

Samples were taken at the beginning, first day, third day, and final on the sixth day. Every sample was centrifuged to separate solids from the liquid. Centrifugation was made using a Heraeus Megafuge 16 at 3,500 RPM for seven minutes. Immediately after the separation, the liquid and particulate fractions were characterized: The liquid samples were analyzed for COD, total and volatile solids, carbohydrates, volatile fatty acids (VFA), alcohols, and lactic acid. The solid samples were analyzed for COD, total and volatile solids, Kjeldahl nitrogen, and carbohydrates. The fresh samples were also placed in the automatic device for biogas determinations (see following chapters).

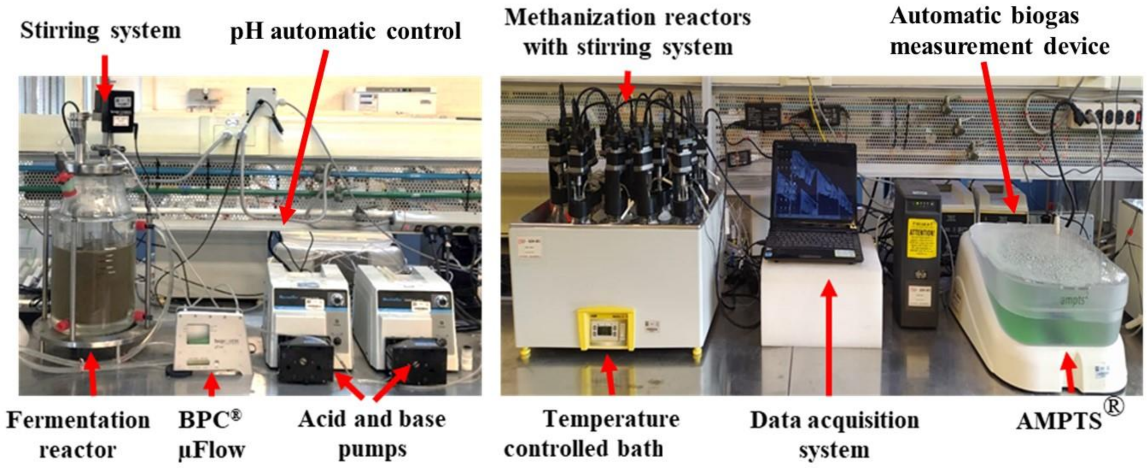

Fig 1. On the left side, experimental set-up with the 4.5 L-reactor, temperature control arrangement, and the pH control system. On the right side, automatic system for biogas measurement AMPTS (Bioprocess Control, Sweden).

OFMSW Sampling and Characterization

The source-separated OFMSW was collected at the Coyoacán transfer station in Mexico City, where sampling was made according to the quartering method (ASTM D5231-92, 2016) [29]. Approximately 120 kg from every one of 11 trucks were separated, and about one ton was thoroughly mixed using a skid-steer loader and shovels. After that, the quartering method was applied two times to reduce the amount to approximately 120 kg. Undesired materials, such as plastic bags, stones, and wood, were hand separated; the remaining "clean" OFMSW was distributed in two-liter freezing bags and frozen at -20 °C. From this amount, 20 kg were overnight defrosted at 4°C, and, using a grain grinder, particles under 5 mm can be obtained [30]. OFMSW was characterized for total solids (TS), 279±8 g/kg, volatile solids (VS), 211±4 g/kg, chemical oxygen demand (COD), 389±48 g/kg, and total Kjeldahl nitrogen (TKN), 5.1±0.4 g/kg. All determinations were made according to Standard Methods [31].

Methanization of the Digestates from Previous Fermentations

For the methanization of the products from OFMSW fermentation, an automatic biogas recording system was used (AMPTS II, Bioprocess Control, Lund, Sweden). This device consists of a 35 °C temperature-controlled water bath with space for 15 reaction flasks (Lauda Aqualine 18). The flasks have a volume of 500 mL with a reaction volume of 400 mL. Every flask is provided with an individual mixing device. Every flask is filled with 8 g VS anaerobic granular sludge from the wastewater treatment plant of a large brewery near Mexico City, 100 mL 0.4 M pH 7 phosphate buffer solution, and tap water to reach 400 mL. To avoid exogenous organic substances, before the tests, the anaerobic sludge was centrifuged (at 3600 RPM 7 minutes) to remove soluble substances, mixed with tap water to the same original volume, and centrifuged again. The TS was adjusted according to the sludge characteristics and humidity (78.12 g_{VS}/kg). One mL of a micronutrient solution was added to every flask for better performance of the microorganisms [24]. The concentrations of the micronutrients are, in mg/L, $FeCl_3 \cdot 4H_2O$, 2000; $MnCl_4 \cdot H_2O$, 2000; $ZnCl_2$, 500; $CoCl_2 \cdot 6H_2O$, 30; $CuCl_2 \cdot 2H_2O$, 50; H_3BO_3, 50; $(NH_4)6Mo_7O_2 \cdot 4H_2O$, 90; $NiCl_2 \cdot 6H_2O$, 50; EDTA, 1000. Nitrogen gas was allowed 30 seconds to remove air from the head volume in every flask to guarantee anaerobic conditions. The biogas production is recorded using a standard computer.

The substrate concentrations used for the methanization test were 25, 50,100, and 15 mL for the liquid digestates and 4, 8, 16, and 24 g (wet weight after centrifugation) for the solid digestates.

Analytical Methods

Dissolved COD (filtering the sample through 0.45 µm membrane) and pH were determined according to Standard Methods [31]. Lactic acid was determined using the spectrophotometric method proposed by Borshchevskaya et al. [32]. Methanol, 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 at a flow rate of 2 mL/min. The injector and detector temperatures were the same, 220°C. The oven temperature ramp was programmed from 40 to 220°C at 10°C/min. The sample was previously filtered using 0.22 µm cellulose filters, and the injected volume was 0.1 ml.

Carbohydrates were determined using phenol and hydrazine sulfate at 490 nm, the so-called colorimetric Dubois Method [33]. This method quantifies total carbohydrates, and, in the case of the liquid fraction, only the soluble ones can be determined.

The biogas composition (CO_2 and CH_4) was determined using a gas chromatograph (SRI 8610c) equipped with a thermal conductivity detector and stainless steel column packed with silica gel (8600-PK1A), helium as carrier gas with a flow of 15.7 mL/min. The oven and detector temperatures were 50 and 150 °C, respectively. The volume of the sample injected was 5 ml.

Kinetic Model

The Michaelis & Menten (M&M) model proposes an enzymatic reaction where the enzyme-substrate, ES, compound can degrade into the initial components, enzyme, E, plus substrate, S, or form a product releasing the free enzyme (Eq. 1). The kinetic equation (Eq. 2) allows simple calculations based on the initial substrate concentration [34, 35]. This model is essential when the substrate concentration and reaction time cannot be determined.

$$E + S \rightleftharpoons ES \longrightarrow P + E \qquad \text{(Eq. 1)}$$

$$v = \frac{\mathrm{Vmax} \cdot \mathrm{S}}{\mathrm{Km} + \mathrm{S}} \qquad \text{(Eq. 2)}$$

Where

v	Reaction rate (NmL/L·d)
Vmax	Maximal possible reaction rate (NmL/L·d)
S	Substrate concentration (g_{COD}/L)
Km	M&M constant (g_{COD}/L)

The experimental results can be adjusted using the linearization proposed by Lineweaver & Burk to obtain the kinetic parameters Vmax and Km [34, 35]. Plotting 1/v vs. 1/S results in a straight line where Km/Vmax is the intercept and 1/Vmax, the inverse value of Vmax (Eq. 3).

$$\frac{1}{v} = \frac{Km}{Vmax} \cdot \frac{1}{S} + \frac{1}{Vmax} \qquad (3)$$

RESULTS AND DISCUSSION

OFMSW Fermentation

From every run in the reactor, samples were taken and characterized at the beginning (time=0) and after 1-, 3-, and 6-days reaction (fermentation) time. It was essential to know how the substrate changed composition according to pH and fermentation time (FT). The samples were placed in a centrifuge to separate the liquid from the solid fractions, and they were characterized and, separately, exposed to methane-producing microorganisms. The black dots in Figure 2 indicate the sum of soluble and particulate COD fractions, carbohydrates soluble in water, and volatile solids (VS) in the soluble and particulate fractions of all digestates. Standard deviation bars are also shown as part of the black dots. The blue bar shows the percent of the liquid fraction in every sample, and the orange indicates the percentage of the particulate fraction.

COD. The preparation of the samples caused the difference in the initial COD of the different pH cases: COD of the pH 6 case was the highest, and the lowest was for pH 4. For all three cases, COD did not change significantly over time. The initial values changed slightly over time, always within the standard deviations (Figure 2). Important to show are the ratios between soluble and particulate COD. For all pH values, the solid fraction represents more than 50% of COD, and this fraction tends to decrease over time as soluble COD increases. At the beginning of every test, solids COD presented values between 70 and 80%, reaching 53 to 59% on the sixth day. COD of the solids hydrolyzed to become part of the liquid COD in one to three days of fermentation time.

Carbohydrates. Total carbohydrates tend to decrease with time in all cases (Figure 2). The start (470-500 g/kg_{VS}) and final (115-130 g/kg_{VS}) values are similar among them in all three cases. At pH 4, the decrease is slightly higher than for the other two pH values: 80% for pH 4, 64% for pH 5, and 73% for pH 6. Carbohydrates decrease due to hydrolysis and fermentation, transforming them into VFA, lactic acid, and alcohols [27, 36]. At time zero, carbohydrates are between 48 and 55% in the liquid, similar to the research of Alibardi and Cossu [37] and Castellón-Zelaya & González-Martínez [38], who reported that between 40 and 60% of carbohydrates are in the liquid from food wastes and OFMSW. These authors also observed that 80% of carbohydrate removal could be achieved at pH 5 in a one-day fermentation. The fastest carbohydrate consumption occurs during the first three days, more drastically at pH 5. According to Ballesteros et al. [16], Balat [39], Taghizadeh-Alisaraei et al. [40], and Yan et al. [41], during the first days, hydrolytic processes are more active than the fermentation ones. Figure 2 indicates that the fractions relationship changes as the readily available soluble substances are consumed.

Volatile solids. Volatile solids follow a pattern like carbohydrates: They decrease rapidly during the first three days, and then they slowly continue falling until day 6 (Figure 2). At pH 4, in the beginning, solids represent 75% and liquid 25% of the VS; the solids hydrolyzed, decreasing to 67% on days one to six. At pH 5, VS hydrolyzed from 77% at the beginning to 66% on the subsequent days. At pH 6, in the beginning, OFMSW has 80% solids decreasing with time to a minimum of 63% on day three, without significant change on day six. Overall, the significant VS hydrolysis took place during the first day of the fermentation. According to Komemoto et al. [21], during the first 48 hours of OFMSW fermentation, carbohydrates are primarily hydrolyzed, followed by lipids and proteins the subsequent days, the least being fibers rich in lignocellulosic compounds.

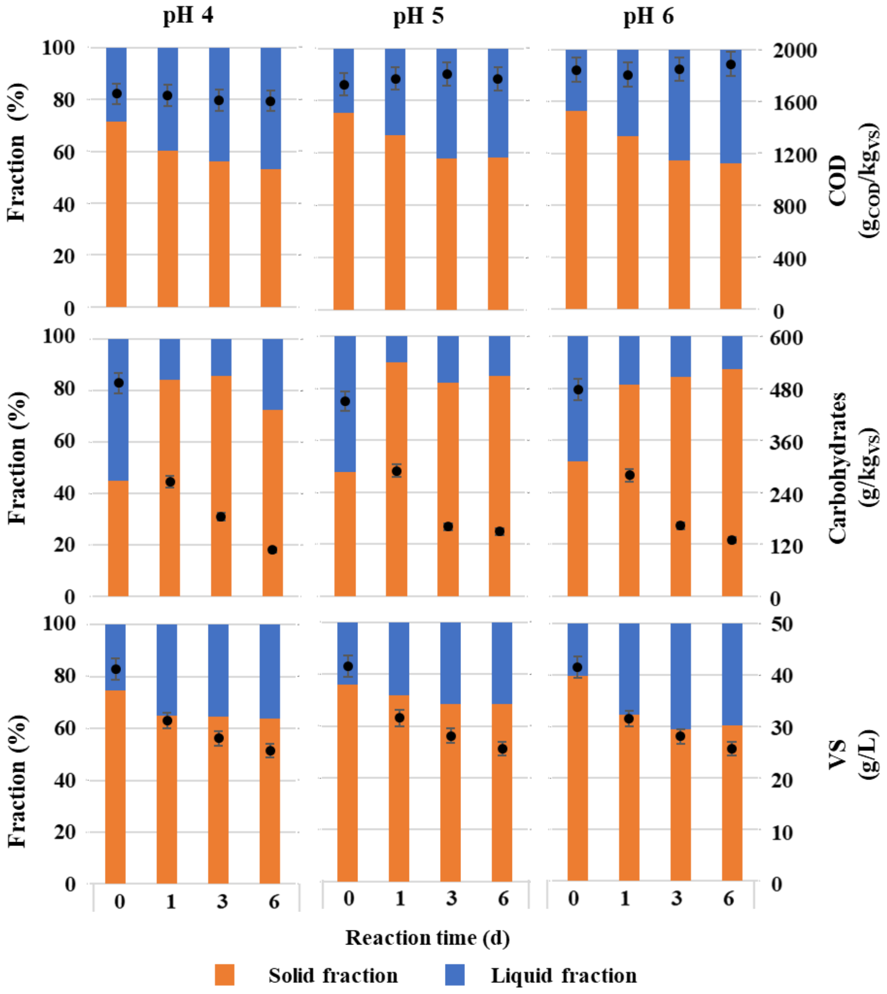

Fig 2. Distribution of COD, carbohydrates, and volatile solids among liquid and solid fractions over reaction time.

Characteristics of the Solid Fractions

Table 1 presents COD, VS/TS rate (TS, total solids), and the hydrolyzation rates for every fermentation time and pH value. For all cases, COD removal increases with the reaction time.

Volatile solids removal was 21% for pH 4, 28% for pH 5, and 24% for pH 6. Although VS was removed, no tendency in the VS/TS ratio can be observed. Although VS was removed (Figure 1), the changes in the VS/TS ratios show no tendencies related to pH or fermentation time (Table 1). Zhang et al. [42], Yan et al. [41], and Hafid et al. [43] worked with fruits and vegetable wastes from a kitchen to produce ethanol and VFA under solids concentrations between 5 and 8%; they found that the highest solids removal was at pH between 5.5 and 6.5. Although Hafid et al. [43] report that lower solids removal rates were observed under pH 4 and reaction times of 8 days, this research proved the opposite because higher solids removal rates were obtained under pH 4 and fermentation times of 6 days.

Table 1. Characterization of the **solid or particulate fractions** from fermentation at different pH values and fermentation times. The hydrolysis rates correspond to the difference between final (day 6) and initial COD values.

pH	Fermentation time (d)	COD (g/kg_{VS})	VS/TS	Hydrolysis rate ($g_{CODsol}/kg_{VS} \cdot d$)
4	0	1290	0.88	NA
	1	1224	0.85	240
	3	1118	0.82	87
	6	1196	0.86	29
5	0	1328	0.88	NA
	1	1211	0.86	283
	3	1205	0.89	97
	6	891	0.77	44
6	0	1152	0.83	NA
	1	1060	0.83	151
	3	866	0.76	98
	6	902	0.80	16

NA, not applicable

Table 1 shows that the hydrolysis rates decrease with fermentation time at different pH values for all cases. On day zero, the substrate has higher concentrations of readily biodegradable substances, and as the reaction times increase, these substances dissolve remaining mostly non-readily hydrolyzable substances [44].

The highest hydrolysis rates were observed at pH 5, pH 4, and pH 6 showed the lowest of all. Lower pH values inhibit microbial metabolism causing slower reaction rates or complete inhibition [45, 46]. Other researchers point out that pH values under 5.5 cause acid reactions, increasing the hydrolysis rates [47, 48, 49]; their recommendation is to ferment at pH values of 5 to 5.5 for better results.

Characteristics of the Liquid Fractions

Table 2 presents the COD in the liquid and water-soluble carbohydrates. The values at time zero correspond to OFMSW suspended in water at 4% TS after 5 minutes under continuous stirring. All samples were centrifuged, and, for "soluble", the sample passed a 0.45 µm membrane filter. After centrifugation, between 80 and 97% COD is soluble, and the difference represents non-soluble substances. Non-soluble COD decreased due to hydrolysis; soluble COD increased with time, mainly during the first three days. Water-soluble carbohydrates rapidly decreased by about 85% during fermentation day one from initial values near 12 g/L. Comparing the values in table 2 with the ones in Figure 2, independently of the pH, the carbohydrates in the liquid fraction decrease rapidly during the first fermentation day. Table 1 shows that the solid fraction undergoes noticeable hydrolysis after the third fermentation day as the COD values decrease.

Unlike the solids in Table 1, the SV/TS ratio in the liquid did decrease with time, mainly during the first fermentation day; this is caused by hydrolysis of the readily biodegradable substances, also represented by increasing total COD, mainly because increasing soluble COD (Table 2). Independently of the pH, the ratio of carbohydrates to soluble COD is 0.62±0.02, which is a relatively high value compared to 0.4 to 0.75 reported by Dahiya et al. [13], Moncada et al. [50], and Hassan et al. [51] for lignocellulosic biomass. From Table 2, it can be calculated that this ratio decreases to values of 0.05±0.01, indicating that, independently of pH, carbohydrates were hydrolyzed and used during fermentation.

Table 2. COD, and carbohydrates in the **liquid fraction** of the fermented OFMSW.

Fermentation conditions		COD	Soluble COD	Non-soluble COD	Water-soluble carbohydrates	VS	VS/TS
pH	FT	g/L	g/L	g/L	g/L	g/L	-
4	0	21	19	2	12.3	11	0.77
	1	27	19	7	1.8	14	0.63
	3	28	27	1	1.1	14	0.61
	6	27	26	1	1.1	13	0.67
5	0	21	19	2	11.7	12	0.87
	1	29	27	2	1.4	14	0.64
	3	28	27	1	1.1	12	0.52
	6	28	25	3	0.8	12	0.59
6	0	23	20	3	12.0	11	0.74
	1	24	23	1	2.1	14	0.58
	3	28	27	1	0.9	15	0.61
	6	27	24	2	0.5	13	0.54

FT = Fermentation. "Total" is after centrifugation; "Soluble" passed a 0.45 µm membrane filter

Metabolites Produced During Fermentation

Figure 3 shows the different metabolites identified during the fermentation under different pH values at different fermentation times, soluble COD (filtered through 0.45 µm, and the fermentation rate. The fermentation rate is the ratio of the metabolites, as COD, over the soluble COD, expressed as a percent. It needs to be considered that the values at time=0 correspond to fresh OFMSW, under the established conditions. More metabolites are released spontaneously at higher pH values.

Solubilization of COD occurs as indicated by increasing values, reaching a maximum after one- and three-days fermentation time. Also, in all three cases, COD decreases on the sixth day. The fermentation rates increased rapidly during the first fermentation day; the final rates increased with pH, from 70% at pH 4 to 93% at pH 5, reaching almost 100% at pH 6.

pH 4. Ethanolic fermentation took place during the first day; for days 3 to 6, it decreased to allow acetic and lactic fermentation to increase with time drastically. These processes can be related to lactic acid acetogenesis [19]. The highest COD value for the sum of all detected metabolites was 18 g_{COD}/L. The difference in total COD corresponds to other soluble substances not considered metabolites of interest.

pH 5. The ethanol concentration observed minor changes over the entire fermentation time, and lactic acid is produced rapidly during the first day to decrease drastically on days 3 and 6. Most of the lactic acid is transformed into butyric and acetic acids. Traces of methanol and propionic acid can be observed during the first and third days.

pH 6. Ethanol, acetic and butyric acids are rapidly produced on the first fermentation day. Ethanol and acetic acids decrease while butyric acid increases dramatically during the third day. Ethanol continued falling with butyric acid on the sixth day to increase acetic and lactic acids. This can be explained through acetogenesis [19].

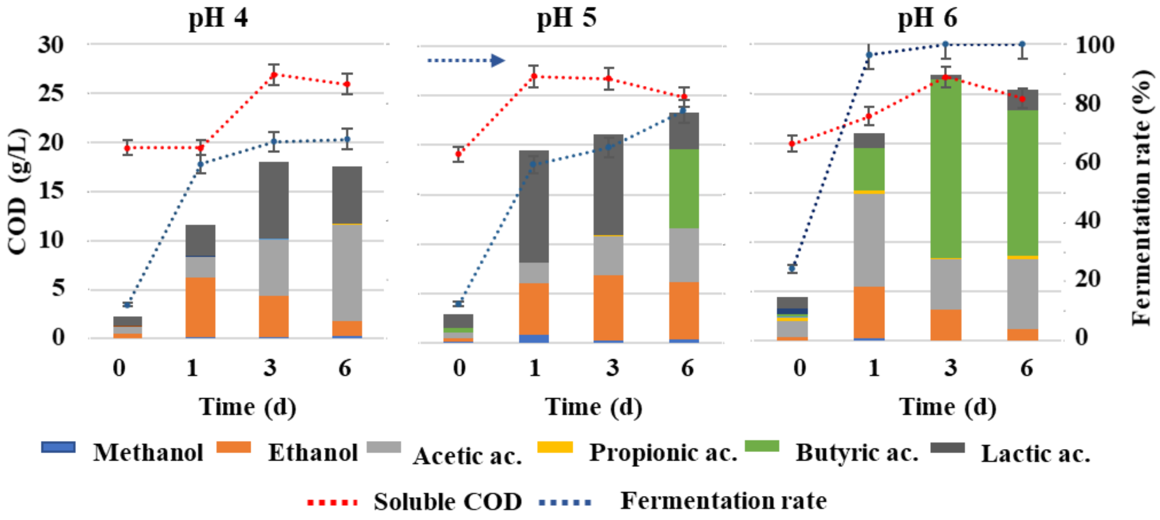

Fig. 3 Metabolites produced during fermentation under different pH values. Comparison of soluble COD with the fermentation rate (soluble COD/COD from all metabolites times 100).

Zheng et al. [11] evaluated the fermentation of vegetables and fruits under pH values of 4, 5, and 6 for 48 hours at 35°C. They did not determine lactic acid, and they concluded that under a pH of 4, ethanolic fermentation predominates with 95% ethanol and 5% acetic acid. Under a pH of 5, the ethanolic fermentation starts fast, and a few hours later, acetogenesis takes place, producing large amounts of acetic acid while decreasing ethanol; as a consequence of acetogenesis, propionic and butyric acids are also produced in smaller quantities [19]. Under pH 6, Zheng et al. (2015) [11] observed ethanolic and acetic fermentation at the beginning, but afterward, butyric acid formation predominated over ethanol and acetic acid.

Zheng et al. [11], Tang et al. [52] evaluated food fermentation under mesophilic temperature and pH values of 4, 5, and 6 for 12 days; unfortunately, they did not report ethanol production, and they concluded that, under pH 6, butyric fermentation predominates over acetic. This research observed the butyric formation under pH 6 and after three days of fermentation time. Unlike this research, Tang et al. [52] report a semi-constant concentration of lactic acid over 10 days under a pH of 5. Figure 2 shows that, under a pH of 5, lactic acid is produced during the first three days, and then it is transformed mainly into butyric acid through acetogenesis. These results agree with Tang et al. [52] as they report that lactic acid fermentation does not settle rapidly and that it drastically increased from day 4 until day 8, reducing acetic acid fermentation rates. They conclude that the best pH for lactic acid production from food wastes is 5.

Methane Production from Liquid Digestates

Figure 3 shows the methane production curves from the liquid digestates resulting from the fermentation at different pH values. The liquid substrate was added at 25, 50, 100, and 150 ml in the 500 mL flasks, where inoculum (washed UASB sludge), micronutrients, and buffer solution were used to complete the 400 mL reaction volume. The inoculum, buffer solution, and micronutrients were the same in every reaction flask. The digestates resulted in different volatile solids concentrations; the need to process the samples immediately after extracting them from the reactor required the initial substrate adjustment in the methanization flasks according to volume. The corresponding VS concentrations are indicated in every curve in Figure 3.

Methanization of the digestates from the fermentation at ***pH 4***

- Independently of the adjusted initial substrate concentration, not one single curve shows a diauxic behavior, indicating that the substrate was simple and readily transformed into methane.
- For the sample after one-day fermentation, the initial slopes are lower than those of the samples of 3 and 6 days.

- The curves corresponding to the lower initial substrate finish the overall methane production in less than one day.
- Independently of the fermentation type or reaction time, after 14 days of methanization, the final methane production is similar among all samples.
- The highest initial methane production (less than 36 hours) corresponds to the sample fermented for 3 days. During the first 36 hours, 75 to 85% of the total methane was produced.

Methanization of the digestates from the fermentation at ***pH 5***

Independently of the fermentation time, the methane production did not stabilize in 14 days for the higher initial substrate concentration.

- The higher lactic acid concentrations negatively affected methane production (compare Figure 3).
- As the lactic acid concentration decreased at 3 and 6 days of fermentation time, the methanization stabilized on day 14.
- Although no diauxic behavior can be observed for the higher initial substrate concentrations, almost constant methane production is evident in a second stage before reaching a final and stable value.
- At pH 5 and 3 days of fermentation, approximately 57% of the total methane was produced.

Methanization of the digestates from the fermentation at ***pH 6***

- Like the curves for pH 5, a second step can be observed for the higher initial substrate concentrations. The first step is a fast reaction producing methane; the second step is slower, and the third step produces methane rapidly before reaching stability.
- The second step can be related to acetogenesis as the methane production is low, and the third step corresponds to methane production from the acetogenesis (compare Figure 3).
- The curves for fermentation times 3 and 6 days are similar in form and methane production.
- At pH 6, selectivity for butyric acid can be observed, reflected as methane production [19].
- At the higher concentrations, the second step corresponds to acetogénesis of butyric acid and the third to methanisation of the products from acetogénesis (compare Figure 3).

Kvesitadze et al. [53], Begum et al. [54], and Lavagnolo et al. [55] worked with OFMSW fermentation-methanization and evaluated the effects of pH on fermentation from 5 to 11. They conclude that neutral pH values are adequate for fermentation with the highest COD conversion rates for hydrogen production, and the best pH values for subsequent methane production are from 5 to 5.5. These values can be compared to those presented in this research, as the fermentation rate decreased with pH, and methane was produced with only one stage (Figure 3). Figure 3 shows how, with decreasing pH values, the diauxic behavior became less noticeable, and the methane production in the first methanization step delivers the higher methane volumes.

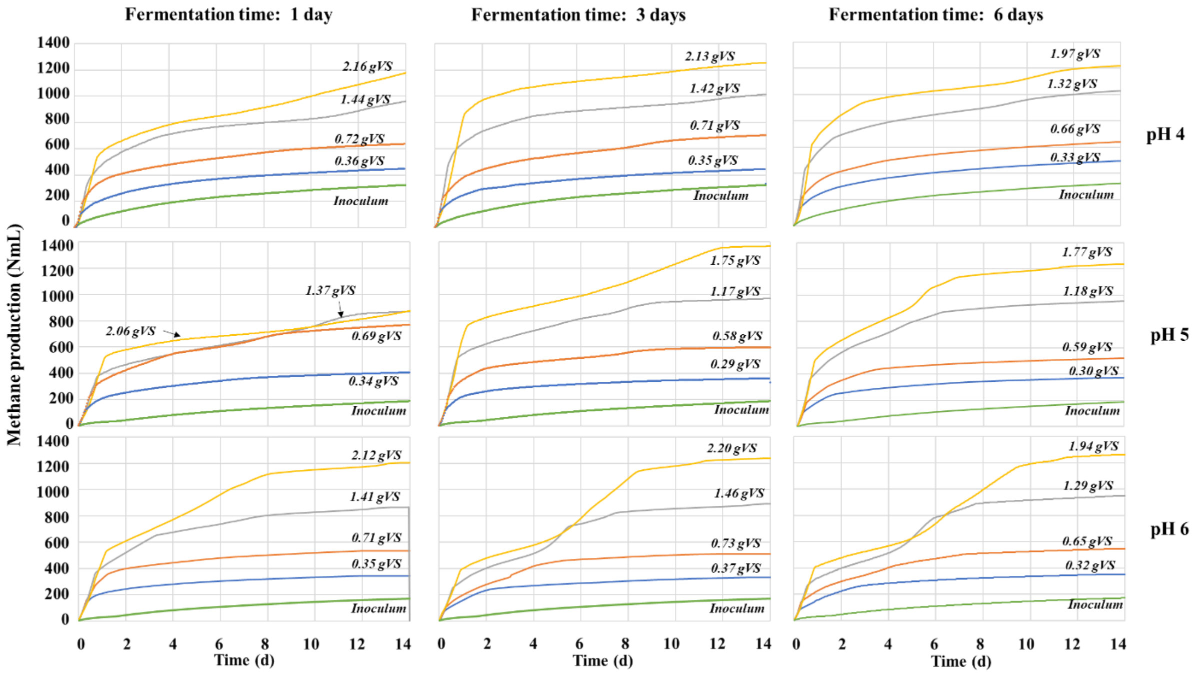

Fig 3. Methane production from the liquid fractions of fermented digestates at different pH values and fermentation times. The liquid digestates were added as a volume in the reaction flasks, and the corresponding VS concentration is indicated in every curve.

Methanization of Solid Digestates

Figure 4 shows the methane production curves from the solids, or particulate, digestates resulting from the fermentation at different pH values. The solid substrate was added at 4, 8, 16, and 24 g as wet mass; as the fermentations resulted in slightly different solids concentrations, Figure 4 shows the resulting VS as initial substrate mass on every curve. As these values were placed in 400 mL, the concentrations can be calculated by simply dividing the values by 0.4 to get them as g_{VS}/L.

Differently from the liquid digestates or fractions, the solid digestates were exposed to methanization for 28 days because the hydrolysis of complex carbohydrates requires longer times [56, 57]. A general and essential observation is that the methanization of the solid fractions showed all diauxic behavior, indicating that, during the first methanization stage, readily biodegradable substances were used for methane production and that, during the second stage, slower hydrolysis processes were present before methanization took place.

Methanization of the digestates from the fermentation at ***pH 4***

- Differently from the liquid digestates, solid digestates present different initial production rates according to the substrate concentration.

- For fermentation times 1 and 3 days, the final methane production is similar. Only the production during the first six days is different for the curves at fermentation times of 3 days.
- The highest methane production was reached with a fermentation time of 6 days.

Methanization of the digestates from the fermentation at ***pH 5***

- At pH 5 a diauxic behavior can be observed for most of the curves.
- The final methane production of the two lower substrate concentrations is similar for the three different fermentation times.
- The two higher initial substrate concentrations show the same behavior during the first 6 days of methanization, corresponding to the first methanization stage. The final methane production is higher for the fermentation time of 6 days.
- The second methanization stage begins between days 8 and 10.

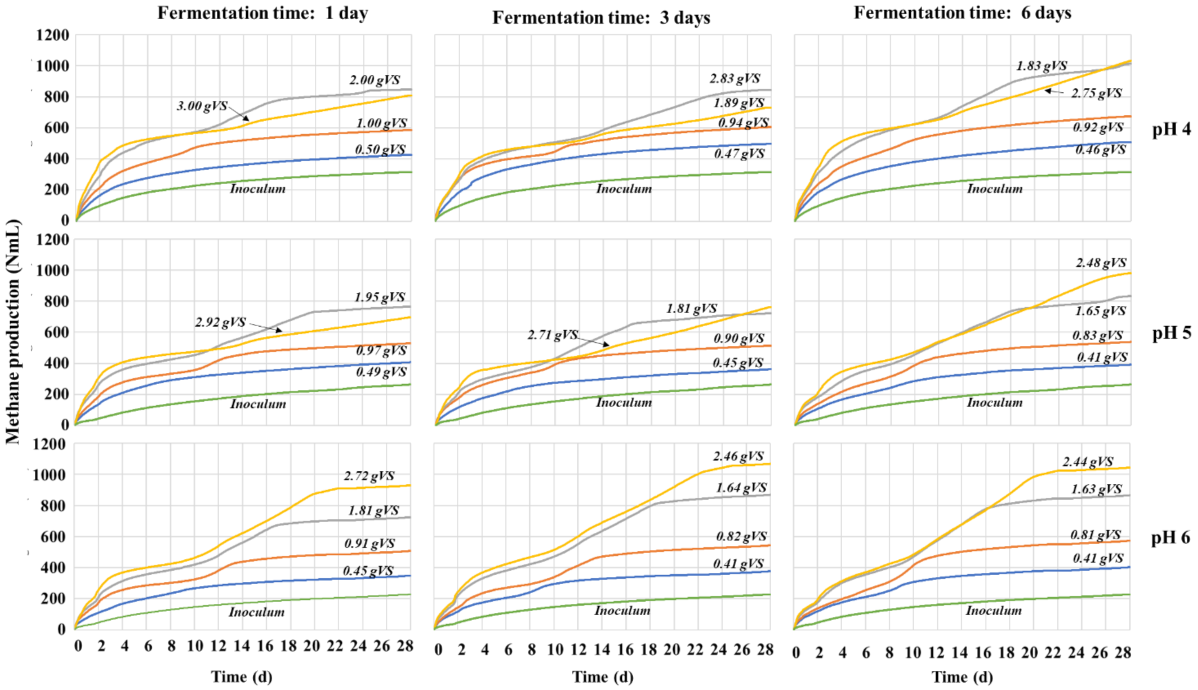

Fig 4. Methane production from the solid fractions of fermented digestates at different pH values and fermentation times. As the digestates were quantified as wet mass in the reaction flasks, exact weighing was not possible; therefore, the corresponding VS initial concentrations are indicated in every curve.

Methanization of the digestates from the fermentation at ***pH 6***

- Independently of the pH and fermentation time, the digestates from the fermentation under this pH report the highest methane production.
- The lowest methane production corresponds to the shorter fermentation time and the highest to the largest fermentation time.

- The separation of the first and second methanization stages is clear, and the final values are considered when they run parallel to the inoculum or blank.

In the following chapters, the analysis of methane production is analyzed in detail.

Calculation of Specific Methane Production (SMP)

Table 3 presents the values resulting from plotting the final methane production (Figures 3 and 4) vs. initial substrate concentration for both liquid and solid fractions. The slopes of the resulting adjusted lines correspond to the specific methane production (SMP) for every pH value and fermentation time. The intercept (substrate concentration = 0) represents the methane production of the inoculum. The reference-specific methane production is the one for the unfermented OFMSW (fermentation time = 0), resulting in 353 NL/kg$_{VS}$.

Without exception, the liquid digestates produce more methane than the solid ones (Table 3) and more than OFMSW without previous fermentation. The highest SMP corresponds to the liquid fraction fermented at pH 5 and the lowest to pH 4. Except for the fraction at pH 6 and fermentation time 6 days, all the SMP for the liquid fraction are higher for higher fermentation times. This reflects the time requirements for hydrolysis and the inhibition caused by acetogenesis of lactic acid at pH 5 and butyric acid at pH 6 (Figure 2) [19].

The SMP increased with fermentation time for the solid fractions at all different pH values. The highest SMP was for fermentation time 6 days and pH 4, and the lowest for fermentation time 1 day at pH 5 (Table 3). At pH 5, lower SMP was observed than at pH 4 and 6. Sawatdeenarunat et al. [58] evaluated 15 different lignocellulosic biomasses (OFMSW, rice, maize, wheat straws, and others) and observed SMP between 41 and 310 NL/kgVS without any pretreatment. Comparing the results of Sawatdeenarunat et al. [58] with the ones of this research, the fermented digestates deliver higher SMP, near the highest reported value of 310 NL/kgVS. Although the liquid fractions yielded higher SMP, the solid fractions also produced higher methane.

The liquid fraction corresponds to the soluble substances contained in the fermented OFMSW, and the values indicated in Table 3 reflect only the specific methane production from the liquid and not the proportional fraction of OFMSW. This will be commented on in detail in the next chapter.

Table 3. Specific methane production after plotting the final methane production, as volume, versus the supplied substrate, as volatile solids, for every pH tested at different fermentation times. The slope is the **specific methane production**, and the intercept corresponds to the ideal inoculum methane

production. The reference value for specific methane production is for unfermented OFMSW, 353 NL/kg$_{VS}$.

Fermentation		Liquid fraction (soluble)		Solid fraction (particulate)	
		SMP	Corr. Coeff.	SMP	Corr. Coeff.
pH	FT	NmL/g$_{VS}$	-	NmL/g$_{VS}$	-
4	1	397	0.9801	247	0.9974
	3	495	0.9478	247	0.9994
	6	456	0.9801	367	0.9999
5	1	409	0.7746	245	0.9999
	3	698	0.9966	291	0.9906
	6	603	0.9920	299	0.9762
6	1	483	0.9996	251	0.9935
	3	500	0.9996	340	0.9865
	6	572	0.9966	316	0.9828

SMP, Specific methane production; FT, Fermentation time

In previous studies on anaerobic digestion of Mexico City's OFMSW, Campuzano and González-Martínez [24] determined the SMP of 541±14 NL/kg$_{VS}$, a value higher than the one of 353 LN/kg$_{VS}$ determined in this research. A significant difference between these two results is the particle size after grinding the OFMSW: This research used 8 mm average particle size, and Campuzano and González-Martínez [24] used 3 mm as the average. With also Mexico City’s OFMSW, but collected at different times, other authors used 8 mm particles after grinding and obtained values of 339 and 318 NL/kg$_{VS}$, similar to the one in this research [39, 59]. Castellón-Zelaya and González-Martínez [39] report the value of 418 NL/kg$_{VS}$ of OFMSW after four weeks of ensiling under anaerobic conditions. According to the same last authors, with increasing substrate concentration in the test flasks, methane production decreases caused by substrate inhibition. When a phosphate buffer was used, they tested substrate concentrations from 1 to 10 g$_{VS}$/L.

Mass Balance to Calculate the Proportional Methane Production in Digestates from Fermented OFMSW

To calculate the specific methane production (SMP) for all the fermented fractions, a mass balance is proposed in equations 4 to 6. Based on volatile solids, the objective of this mass balance is to determine the contribution to methane production of the liquid and solid fractions for every case considering pH and fermentation time. The mass balance results are then related to the specific methane production (Table 3) to calculate the corresponding methane production from every fraction.

$$M_{TVS} \cdot SMP_T = m_{LVS} \cdot SMP_L + m_{SVS} \cdot SMP_S \qquad \text{(Eq. 4)}$$

Where

M_{TVS}	Total volatile solids
SMP_T	Total specific methane production
m_{LVS} and m_{SVS}	Mass of volatile solids in the liquid and solid fractions

SMP_L and SMP_S Specific methane production in the liquid and solid fractions

Rearranging equation 1

$$SMP_T = \frac{m_{LVS}}{M_{TVS}} \cdot SMP_L + \frac{m_{SVS}}{M_{TVS}} \cdot SMP_S \qquad \text{(Eq. 5)}$$

Considering $\frac{m_{LVS}}{M_{TVS}} = f_L$ and $\frac{m_{SVS}}{M_{TVS}} = f_S$;

Where f are the fractions of volatile solids in the liquid and solid samples. Equation 3 results in the general mass balance.

$$SMP_T = f_L \cdot SMP_L + f_S \cdot SMP_S \qquad \text{(Eq. 6)}$$

Table 4 shows two main sets of results: 1) Methane production for the first methanization stage (compare Figures 3 and 4), and 2) total methane production, considering the first and second stages. A column shows the contribution of the first stage over the total methane production, indicating values from 49 to 69%. The contribution increased with decreasing pH. The first stage required 2 to 4 days of methanization for the liquid fraction and 6 to 8 days for the solid fraction (Figures 3 and 4).

The highest SMP is related to pH 5 in liquid and solid fractions. In the first stage, the liquid fraction produced methane between 68 and 132 NL/kg$_{VS}$, without showing a tendency related to pH. Methane production from the solid fraction oscillated between 91 and 149 NL/kg$_{VS}$, and no trend can be identified related to pH. The combined methane production (sum of liquid and solid fractions) shows the lowest value of 181 NL/kg$_{VS}$ under pH 5 and fermentation time one day; the highest value of 272 NL/kg$_{VS}$ contrasts with the lower ones, it corresponds to pH 5 and 3 days fermentation. The following lower values of 206, 207, and 209 correspond to different pH at different fermentation times. In short, for the liquid fractions, no tendencies could be found on how SMP and pH are related.

Total specific methane production of the solid fractions, as expected, is higher than the one for the first stage; in the column "% of first stage over final" the percentage of the first step is indicated for all cases. For the liquid and solid fractions, SMP increased from fermentation day 1 to 3, and no significant changes can be observed in fermentation from day 3 to 6. For liquid fractions, the lowest was 115 NL/kg$_{VS}$ for pH 5 and one-day fermentation; the highest value was 227 NL/kg$_{VS}$ for pH 6 and 6 days fermentation. For the solid fractions, the lowest value is 159 for pH 4, fermentation time 3 days, and the highest value is 234 NL/kgVS for pH 4 and fermentation time 6 days. Overall, no tendencies could be observed for SMP of liquid and solid fractions.

The total SMP increases with fermentation time and the highest values correspond to higher pH values. The last column in Table 4 indicates how much methane was produced when compared to unfermented OFMSW (reference value). The lower fermentation times report negative values, meaning that fermentation for one day produces less methane than unfermented OFMSW. The highest value was 19% for pH 5 fermentation time 3 days and 18% for pH 6 and fermentation time 6 days.

Bacab et al. [25], Li et al. [27], Majhi and Jash [28], Lindner et al. [57], and Shen et al. [60] propose to separate the liquid from the solids after OFMSW fermentation and perform anaerobic digestion separately. Working with fermented fruit and vegetable wastes and wastes from a restaurant, they report overall higher methane production from 35 to 50% over unfermented wastes; if both liquid and solid fractions are exposed to anaerobic digestion together, an increase in methane production of only 10 to 30% can be obtained [56, 61]; they also report that for the unfermented wastes, the required time for methane production required 22 to 40 days, and with previously fermented wastes, the time decreased to 10 to 18 days. This work shows that when only the liquid fraction is exposed to anaerobic digestion, the required time to finish the methane production reaction is from 2 to 6 days; when OFMSW is exposed to anaerobic digestion, the time to complete the reaction is from 22 to 35 days.

Fongsatitkul et al. [56] worked with fruit, vegetable, and other food wastes. They analyzed the methane production curves, concluding that the first methanization step produced between 50 and 60% of the total methane, corresponding to a reaction time reduction of 75 to 80% of the required for the complete curve to finish.

The overall methanization time can be reduced with high methane production rates if only the first methanization stage is considered for methane production. As reaction times decrease, the reactor volumes decrease. A compromise can be reached when fast methane production is required to cover electricity consumption at specific times. This research concludes that shorter methanization times of 2 days for liquid digestates and 8 days for solid digestates are adequate for faster methane production (Table 4).

Kinetic Analysis of Methane Production

As previously stated, shorter methanization times can be critical in cases where methane is needed for fast energy production. Using the results from the methanization in the first stage, the kinetic parameters of methane production from fermented digestates are analyzed using the Michaelis & Menten model (Eq. 2) through the linearization proposed by Lineweaver and Burke (Eq. 3), Table 5 presents the kinetic parameters Vmax and Km for the liquid and solid fractions according to pH and fermentation time.

Table 4. Specific methane production from the liquid and solid fermented digestates from OFMSW at different methanization stages, and their relative importance. Consult Figures 3 and 4 to identify the stages. The liquid fraction presented only one stage as no diauxic behavior was observed. Unfermented OFMSW has a specific methane production of 353 NL/kg_{VS}.

				Methane production in first stage			Methane production at the end of second stage		
pH	FT		Fraction (as VS)	% of first stage over final	CH_4 production (NL/kg_{VS})	CH_4 production (combined) (NL/kg_{VS})	CH_4 production (NL/kg_{VS})	CH_4 production of both fractions (NL/kg_{VS})	% Increase above unfermented OFMSW
4	1	Liq	0,35	59±4	83	207	139	319	-9
		Sol	0,65	69±3	124		180		
	3	Liq	0,35	75±6	132	237	176	335	-5
		Sol	0,65	66±7	106		159		
	6	Liq	0,36	65±4	108	257	165	399	13
		Sol	0,64	64±7	149		234		
5	1	Liq	0,28	59±6	68	181	115	291	-17
		Sol	0,72	64±7	113		176		
	3	Liq	0,31	69±8	151	272	219	418	19
		Sol	0,69	60±9	121		200		
	6	Liq	0,32	53±17	101	206	191	389	10
		Sol	0,68	53±9	105		198		
6	1	Liq	0,35	64±11	109	201	171	333	-6
		Sol	0,65	56±9	91		162		
	3	Liq	0,41	52±14	107	212	206	406	15
		Sol	0,59	53±9	105		200		
	6	Liq	0,40	49±12	112	209	227	418	18
		Sol	0,60	51±9	97		191		

FT, Fermentation time; Liq, liquid; Sol, solid

Table 5. Michaelis & Menten kinetic parameters from the liquid and solid digestates. The Vmax and Km for the unfermented reference OFMSW are 1,428 NmL/L·d·and 5.6 g_{VS}/L, respectively. These values were obtained using Eq. 3 (linearization of Lineweaver and Burk).

		Vmax (NmL/L·d)			Km (g_{VS}/L)			Regression coefficients		
Fermentation time (d)		1	3	6	1	3	6	1	3	6
Liquid fraction	pH 4	2146	2846	2974	0.9	1.1	1.2	0.933	0.957	0.887
	pH 5	1366	2306	2248	0.3	0.8	1.0	0.913	0.972	0.914
	pH 6	1512	1232	1454	0.8	0.5	0.5	0.879	0.985	0.902
Solid fraction	pH 4	717	349	479	5.9	0.7	2.8	0.999	0.958	0.901
	pH 5	414	326	222	2.6	2.2	1.5	0.985	0.989	0.916
	pH 6	479	317	222	5.0	2.5	1.4	0.964	0.982	0.816

For the liquid fraction at pH 4, Figure 6 shows that the Vmax (maximal specific methane production rate) increased with fermentation time. pH 4 and 6 days fermentation time reports the highest Vmax with 2974 NmL/L·d; this value is twice as significant as unfermented OFMSW. For pH 5, Vmax increased from day 1 to 3, and then it remained without noticeable changes until day 6. For pH 6, Vmax decreased and then increased to a value slightly lower than for day 1. As a reference, Vmax for unfermented OFMSW is 1,428 NmL/L·d.

The Vmax values for the solid fractions decreased with increasing fermentation time (Table 5). For pH 4, Vmax decreased from 717 on fermentation day 1 to 349 on day 3, and it increased again to 479 NmL/L·d. For pH 5 and 6, Vmax values decreased steadily from 414 and 479 to 222 NmL/L·d, respectively. All the Vmax values for the solid fractions are significantly lower than those for the liquid fractions. The highest Vmax value is approximately half of the corresponding to the unfermented OFMSW.

Dogan et al. [62] and Fongsatitkul et al. [56] indicate that the liquid fraction, combined or separated from the solids, is essential for methane production as it represents readily biodegradable substances responsible for the first methanization stage (higher Vmax) and that the second methanization stage represents slower hydrolysis processes (lower Vmax).

Table 5 also shows that Km, the Michaelis & Menten proportionality constant, can be related to the affinity of the substrate to the enzyme. Lower Km values indicate higher affinities. For the liquid fraction, all values are lower than 1.2 g_{VS}/L, meaning good affinity of the methanogens for the substrate. For the solid fractions, at the different pH values, all values decrease with fermentation time; this can be interpreted as increasing hydrolysis of the solids producing more available substances for the methanogens as the fermentation time increases. Zhang et al. [11] worked with pretreatment of the OFMSW solid fraction, and they concluded that, after acid pretreatment for 2 to 5 days, the hydrolyzed residues produce more methane (higher Vmax and lower Km) than the sample without pretreatment. Ma et al. [9] and Wu et al. [15] recommend ethanolic and acetic fermentations because they produce readily available substrates for methanogens, increasing the methanization rates. Considering that the Km of unfermented OFMSW is 5.6 g_{VS}/L, all fermented digestates present lower values, especially the ones for the liquid fractions; it can be concluded that fermentation, independently of pH and fermentation time, reduces the complexity of OFMSW and allows better and faster methanization.

CONCLUSIONS

The main conclusions that can be drawn from this work are:

- Lower pH values and shorter fermentation allow better ethanol and lactic acid production. Higher pH values and longer fermentation times promote undesired butyric acid formation.
- During fermentation, hydrolysis mainly occurs during the first day causing total carbohydrates and COD to decrease with time. Hydrolysis rates decrease with fermentation time at all different pH values.
- Without exception, the liquid digestates produce more methane than the solid ones and unfermented OFMSW.
- With decreasing pH, the liquid digestates present only one methanization stage (no diauxic behavior).
- Methanization of the solid fractions showed diauxic behavior: The first stage is methanization of readily biodegradable substances; slower hydrolysis processes and acetogenesis cause the second stage.
- The total specific methane production increases with fermentation time, and the highest values correspond to higher pH values. Previously fermented OFMSW produced 19% more methane than unfermented one.
- The first methanization stage of the liquid fractions requires 1 to 2 days, and the solid fractions from 4 to 8 days. Both fractions together represent 49 to 69% of total methane production.
- From modeling with the Michaelis & Menten model, the Vmax values (maximal specific methane production rates) for the liquid fractions are significantly higher than those for the solid ones. Vmax substantially increases with decreasing pH during fermentation.
- The proportionality constants Km for the liquid digestates are significantly lower than for the solid digestates; lower Km represents good affinity of the methanogens for the readily biodegradable

substrate. Higher Km values for the solid fractions indicate complex hydrolysis processes and acetogenesis before methanization.

- OFMSW fermentation, independently of pH and duration, reduces the complexity of OFMSW and allows better and faster methanization.

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CRediT authorship contribution statement

Investigation, conceptualization, and writing, G.D. Jojoa-Unigarro. Conceptualization, writing, and editing: S. González-Martínez

Declaration of Competing Interest

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

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