



## Effect of incubation conditions on the aerobic pretreatment of the organic solid wastes and the volatile fatty acids production

### Efecto de las condiciones de incubación en el pretratamiento aeróbico de los residuos sólidos orgánicos y la producción de ácidos grasos volátiles

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#### Abstract

In the present work, the effect of an aerobic pretreatment on the hydrolysis and acidogenesis of the organic solid wastes (OSW) was evaluated. The aerobic pretreatment was carried out under three incubation conditions: the first condition was at 30 °C for 24 hours (isothermal), the second condition was at 30 °C for 12 hours followed by 12 hours at 35 °C, and the third condition was at 35 °C for 12 hours followed by 12 hours at 40 °C. The enzymatic activity was analysed during the aerobic pretreatment, and the product obtained was subsequently fermented in anaerobic hydrolytic leach bed reactors (AHLB) to obtain volatile fatty acids (VFA). The best hydrolysis results were achieved with the aerobic pretreatment at 30 °C for 24 hours, obtaining activities of xylanases and pectinases of 115 and 73 U g<sup>-1</sup> dry matter (DM) respectively, after 18 hours and 100 U g<sup>-1</sup> DM of proteases at the beginning of the process; values that are 2.7, 2 and 4 times higher than previously report. Also, a hydrolysis constant (k) of 0.081 d<sup>-1</sup> was obtained, with the productivity of VFA of 0.82gLr<sup>-1</sup>d<sup>-1</sup>, with predominance in acetate, the main precursor of methanogenesis in the final stage of anaerobic digestion.

*Keywords:* Organic Solid Wastes (OSW), aerobic pretreatment, hydrolysis, leachate, volatile fatty acids.

#### Resumen

En el presente trabajo, se evaluó el efecto de un pretratamiento aeróbico sobre la hidrólisis y la acidogénesis de los residuos sólidos orgánicos (RSO). El pretratamiento aeróbico se llevó a cabo bajo tres condiciones de incubación: la primera condición fue a 30 °C durante 24 horas (isotérmica), la segunda condición fue a 30 °C durante 12 horas seguido de 12 horas a 35 °C, y la tercera condición fue a 35 °C durante 12 horas seguido de 12 horas a 40 °C. Se analizó la actividad enzimática durante el pretratamiento aeróbico y el producto obtenido se fermentó posteriormente en reactores hidrolíticos anaerobios de lecho escurrido (RALE) para obtener ácidos grasos volátiles (AGV). Los mejores resultados de hidrólisis se lograron con el pretratamiento aeróbico a 30 °C durante 24 horas, obteniendo actividades de xilanasas y pectinasas de 115 y 73 U g<sup>-1</sup> materia seca (MS) respectivamente después de 18 horas y 100 U g<sup>-1</sup> MS de proteasas al inicio del proceso; valores que son 2.7, 2 y 4 veces más altos que los reportados previamente. Además, se obtuvo una constante de hidrólisis (k) de 0.081 d<sup>-1</sup>, con una productividad de AGV de 0.82gLr<sup>-1</sup>d<sup>-1</sup>, con predominio en acetato, el principal precursor de la metanogénesis en la etapa final de la digestión anaerobia.

*Palabras clave:* Residuos sólidos orgánicos, pretratamiento aerobio, hidrólisis, lixiviado, ácidos grasos volátiles.

## 1 Introduction

The global production of municipal solid waste is approximate of 1.3 billion tons per year and it estimated that in 2025 could increase to 2.2 billion tons per year, with 46% organic content (Al Seadi *et al.*, 2013).

In Mexico, the increasing volume of generated solid waste, the difficulty of its collection, the rapid depletion of sanitary landfills and the waste of useable materials, have made it necessary to establish mechanisms to promote recycling and treatment (SEMARNAT, 2017).

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At present, there are several treatments used to stabilise the organic fraction of municipal solid waste (OFMSW), and one of them is anaerobic digestion; which is biological process that in absence of oxygen results in two products: biogas (composed of methane and carbon dioxide) and a stabilised biosolid. During the last decades, anaerobic digestion of organic matter has been reported as a sustainable technology used for the treatment of organic waste and the production of a renewable source of energy (De Baere, 2004).

The main obstacle in the anaerobic digestion of organic wastes is the hydrolysis stage (Mata-Alvarez *et al.*, 2000). Therefore, several strategies have been reported to improve the hydrolysis of organic matter, which increase process stability and methane yields. These strategies include optimisation of the inoculum-substrate (I/S) ratio (Raposo *et al.*, 2009), pH adjustment (Cysneiros *et al.*, 2008), particle size reduction and the application of thermal, chemical and enzymatic or biological pretreatments (Kim *et al.*, 2003; Fdez-Güelfo *et al.*, 2011a; Lopez-Torres *et al.*, 2008).

A high methane yield has been reported in the anaerobic digestion of agricultural residues in two-stage systems (Xu *et al.*, 2011). The advantage of a two-stage system over the single-stage system is that hydrolysis and methanogenesis can be optimised separately since the first step is regularly the limiting step when the substrate is abundant in complex or difficult compounds degradation.

The introduction of a controlled and limited amount of oxygen into a pretreatment stage or directly during the anaerobic digestion process of OFMSW has improved hydrolysis and methane yields (Ngugyen *et al.*, 2007). This increase has been attributed to the action of secreted enzymes by aerobic bacteria during the aerobic period. Charles *et al.* (2009) reported the effect of pretreatment with aeration for 2 days before anaerobic digestion of the OFMSW in batch conditions, obtaining an increase in methane production with a retention time of 12 days and under thermophilic conditions.

The application of a biological pretreatment before anaerobic digestion of OFMSW has also been reported. This consisted of the addition of mature compost to OFMSW to improve the solubilisation of organic matter during the anaerobic digestion, and improved the organic matter degradation by up to 61% over the control and therefore, increased biogas production (Fdez-Güelfo *et al.*, 2011b). Xu *et al.* (2014) reported recirculation of leachate and micro-aeration, applied to food waste before

anaerobic digestion to improve hydrolysis and methane production, achieving a 60-75% increase in solubilisation efficiency of organic matter during the hydrolysis-acidogenesis phase and an 18% increase in methane yield.

Martínez-Valdez *et al.* (2016) reported that the addition of OFMSW aerobically pre-treated for five days in tubular reactors and OFMSW without treatment with a solids content of 26%, resulting in a net increase of 18 and 39% in methane production and methane yield, respectively.

Ramírez *et al.* (2014) reported the anaerobic digestion of the OFMSW in two stages, the first stage involved hydrolysis-acidogenesis in an AHLB reactor, with a 45% reduction in total solids and a high concentration of VFA in the leachate and a solids retention time of 30 days. In the second stage, the leachate diluted with municipal wastewater was methanised in an up-flow anaerobic sludge blanket (UASB) reactor, obtaining chemical oxygen demand (COD) removal efficiencies greater than 90% and with high methane yields.

Rodríguez-Pimentel *et al.* (2015), reported the effect of the organic load on the anaerobic digestion of OFMSW in two stages. The first stage involved hydrolysis-acidogenesis in an AHLB reactor, operated continuously, obtaining efficiencies in the elimination of volatile solids of up to 70% in a 27 day solids retention time (SRT) and a hydrolysis rate of  $0.037\text{d}^{-1}$ . The leachate generated with high concentrations of soluble COD and diluted with municipal wastewater, at different organic loads were methanised in a UASB, achieving COD elimination efficiencies higher than 87% and methane productivity of  $9\text{ LCH}_4\text{ Lr}^{-1}\text{ d}^{-1}$ .

The objective of this work was to evaluate the effect of an aerobic pretreatment for 24 hours on the hydrolysis of the organic solid wastes (OSW) in three incubation conditions, as a strategy to improve the hydrolysis and acidogenesis of the OSW in anaerobic hydrolytic leach bed (AHLB) reactors, operated in batch for 21 days.

## 2 Materials and methods

### 2.1 Sampling and characterisation of the organic solid wastes

OSW samples (fruit and vegetable wastes: papaya, pineapple, orange, apple, melon, mango, carrot and jicama), were collected from food stores near to

Metropolitan Autonomous University (MAU) Campus Iztapalapa. OSW were characterised based on the content of total solids (TS), volatile solids (VS), total chemical demand of oxygen (COD), total carbohydrates, pH, humidity and density.

## 2.2 Aerobic pretreatment of the OSW

The characterized OSW was inoculated with 8% of mature compost and mixed with other components according to reported by Martínez-Valdez *et al.* (2015), to maintain a C/N (carbon/nitrogen) of 30 and in this way favour growth and metabolic activity based on the results reported in other previous studies (Nattipong *et al.*, 2006; Sudarut *et al.*, 2012).

The aerobic pretreatment of the mixture was carried out in tubular bioreactors (TBR), 5 cm in diameter and 15 cm in height, configuration and operation conditions for feeding were similar to those reported by Martínez-Valdez *et al.* (2016).

The packed TBR's were incubated at three different temperature conditions (T1 at 30 °C, T2 at 30 °C and 35 °C and T3 at 35 °C and 40 °C), until the maximum rate of CO<sub>2</sub> formation was reached, which was decided based on the results previously reported Martínez-Valdez *et al.* (2015). The first condition (TBR<sub>1</sub>) was isothermal at 30 °C; the second (TBR<sub>2</sub>) was maintained at 30 °C for 12 h and, subsequently, at 35 °C for 12 more hours and the third condition (TBR<sub>3</sub>) was incubated at 35 °C for 12 h and, subsequently, at 40 °C for another 12 h. At the beginning and the end of the incubation TS, VS, COD, pH, and total carbohydrates were determined (Table 1). A flow of 0.5 vkgm (volumetric airflow per kilogram of mixture) was applied, then the CO<sub>2</sub> (%) and airflow (mL min<sup>-1</sup>) coming from the TBR were measured, using a respirometry system which enables online analysis of CO<sub>2</sub> concentration and flow measurement in the exhausted dry gases coming from respiration, according to that reported by Martínez-Valdez *et al.* (2016). The calibration of respirometry equipment for measuring gas was performed using CO<sub>2</sub> of a known concentration. The results were expressed in terms of the CO<sub>2</sub> formation rate as mg h<sup>-1</sup> g<sup>-1</sup> dry matter (DM). Total formation of CO<sub>2</sub> was estimated by assessing the area under the curve of CO<sub>2</sub> formation rate by the trapezoid method (Chapra and Canale, 1998).

## 2.3 Enzymatic activities

The enzymatic activities amylase, cellulase, pectinase, and xylanase were quantified by the release of sugars using starch, cellulose, pectin, and xylan Birchwood, respectively, as substrates. The reaction was carried out on a 50 millimolar (mM) citrate buffer, following the proposed method for amylases (Omemu *et al.*, 2005), cellulases (Ghose, 1987), pectinases (Zhang *et al.*, 2000) and xylanases (Pradal-Velázquez *et al.*, 2018; Castañeda-Casasola *et al.*, 2018). Protease activity was determined using a method modified by Alef *et al.* (1995). A 1 mL aliquot of the enzyme extract was added to 5 ml of casein (2% w/v) and incubated at 50 °C under continuous stirring for 2 hours. The enzymatic activities were expressed in U g<sup>-1</sup> dry matter, where a unit U is the amount of enzyme that catalyses the formation of 1 μmol of the product per minute. All analyses were performed at least in duplicate.

## 2.4 Hydrolysis and acidogenesis of waste aerobically pre-treated in anaerobic hydrolytic leach bed (AHLB) reactors

After the aerobic pretreatment, the mixture of the residues was fermented in anaerobic hydrolytic leach bed (AHLB) reactors with a useful volume of 300 mL, and it was decided to use the AHLB configuration mainly to avoid energy expenditure, based on the results reported in other previous works (Ramírez *et al.*, 2014; Rodríguez-Pimentel *et al.*, 2015). Each one of the AHLB reactors was packed with 150 g of the wet mass and inoculated with 10% (w/w) of anaerobic sludge, proceeding from a UASB that treats the wastewater from the campus. The reactors were incubated at 30 ± 2 °C for 21 days (Figure 1). To prevent the soluble compounds (ex. VFA) from fermentation from inhibiting the process, a runoff liquid was added from the top of the reactor, which drained the soluble material to the bottom of the reactor, which had a mesh of 0.3 cm, generating a leached with high concentrations of soluble COD, as reported by Rodríguez-Pimentel *et al.* (2015). The runoff liquid consisted of 10 mL of the UASB reactor effluent that treats the wastewater from the campus (MAU, Campus Iztapalapa) to favour the degradation of the OSW. Each of the treatments was performed in duplicate and with an AHLBc control reactor containing the OSW without the aerobic pretreatment.

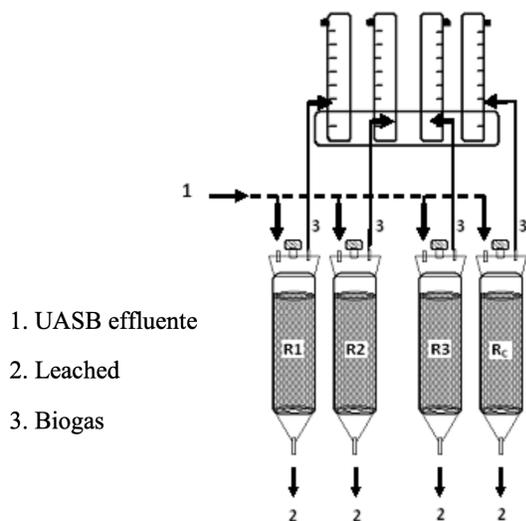


Fig. 1. Anaerobic hydrolytic leach bed (AHLB) reactors: AHLB<sub>c</sub> (R<sub>C</sub>); AHLB<sub>1</sub> (R<sub>1</sub>); AHLB<sub>2</sub> (R<sub>2</sub>); AHLB<sub>3</sub> (R<sub>3</sub>).

Soluble COD as reported by Bautista-Ramírez *et al.* (2018), pH, volatile fatty acids (VFA) and fermentation products in the leachates produced in each AHLB reactors were analyzed.

### 2.5 Mass balance and hydrolysis constant

To determine the operation of the AHLB reactors in batch mode, the mass balance was performed using the following equation:

$$V \frac{ds_1}{dt} = F(S_0 - S_1) - V k(S_0 - S_1) \quad (1)$$

where

- $V$  = Reactor volume (L)
- $S_0$  = Initial concentration (g COD L<sup>-1</sup>)
- $S_1$  = Final concentration (g COD L<sup>-1</sup>)
- $F$  = Leachate flow (L d<sup>-1</sup>)
- $k$  = Hydrolysis constant (d<sup>-1</sup>)
- $t$  = time (d)

From the volatile solids VS balance in the AHLB reactor, the hydrolysis constant  $k$  was obtained from equation (2).

$$V \frac{dS_v}{dt} = W_0 S_{v0} - W_1 S_{v1} - V_L k S_v = 0 \quad (2)$$

$$k = \frac{(W_0 S_{v0} - W_1 S_{v1})}{V_L * S_v} \quad (3)$$

where:

- $V$  = Reactor volume (L)
- $V_L$  = Volume of leachate (L)
- $S_{v0}$  = Initial concentration (g VS L<sup>-1</sup>)
- $S_{v1}$  = Final concentration (g VS L<sup>-1</sup>)
- $S_v$  = Concentration (g VS g<sup>-1</sup> OSW)
- $W_0$  = Initial mass (g OSW d<sup>-1</sup>)
- $W_1$  = Final mass (g OSW d<sup>-1</sup>)
- $k$  = Hydrolysis constant (d<sup>-1</sup>)
- $t$  = time (d)

### 2.6 Analytical methods

The moisture content of the OSW was determined by the gravimetric method based on weight loss after placing the samples at the constant weight in an oven at 60 °C for 24 hours (SEMARNAT, 2017). Dry matter (DM) was determined according to the standard procedures following the Test Methods for the Examination of Composting and Compost (Puyuelo *et al.* 2010). To determine the pH, 1g of the sample was weighed and distilled water was added in a ratio of 1:10 (w/w), stirred for 1 minute and allowed to stand for 1 minute (SEMARNAT, 2017). The pH was measured in the suspension with a potentiometer (Corning pH/ion Analyzer 455) previously calibrated. Total soluble carbohydrates were quantified following the colorimetric method of Dubois (1956) in both the OSW and the leachate. The chemical oxygen demand (COD) was determined by the closed reflux method (APHA, AWWA, WPCF. 2005). Total solids (TS), fixed solids (FS) and volatile solids (VS) were analysed by gravimetry according to standard methods (APHA, AWWA, WPCF. 2005). The volatile fatty acids were analysed on a gas chromatograph (Hewlett Packard series II model 5890), equipped with flame ionisation detector (FID) and Superox FA capillary column, AT 1000, using an injection volume of 2 µL, from a prepared sample (filtrate volume: 950 µL and 50 µL 50% HCl). The operating conditions of the equipment were as follows: column temperature of 120 °C to 140 °C, with an increase of 10 °C min<sup>-1</sup>, injector temperature of 130 °C, detector temperature of 150 °C, and nitrogen was used as a carrier gas at 3 mL min<sup>-1</sup> (Moreno-Cruz *et al.*, 2018).

## 2.7 Statistical analysis

All measurements and tests were carried out in triplicate and the results were expressed as mean  $\pm$  standard deviation. Tukey's HSD test was used to compare the means and to reveal significant differences among samples (at  $\alpha = 0.05$ ). Statistical analysis was performed using SPSS 18.0 software.

## 3 Results and discussion

### 3.1 Aerobic pretreatment of the OSW

Figure 2 shows the CO<sub>2</sub> formation rate (CO<sub>2</sub> FR, mg CO<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> DM) during aerobic pretreatment of OSW conditions under the three assayed conditions. In all three cases, a rapid increase in the CO<sub>2</sub> formation was observed until reaching a maximum around the first 12 h of incubation, which can be explained as a phase of diauxia, due to the consumption of rapidly assimilated substrate. After that, a rapid phase of CO<sub>2</sub> production was observed possibly due to the release of sugars as a result of the hydrolytic enzymes action. In all cases, a maximum of the CO<sub>2</sub> formation rate was observed shortly before 24 h of incubation (22-23 h). Therefore, the decision was made to finish the aerobic pretreatment by 24 h of the process. The time in which the maximum rate of CO<sub>2</sub> formation occurs is similar in other reports (Martínez-Valdez *et al.*, 2015).

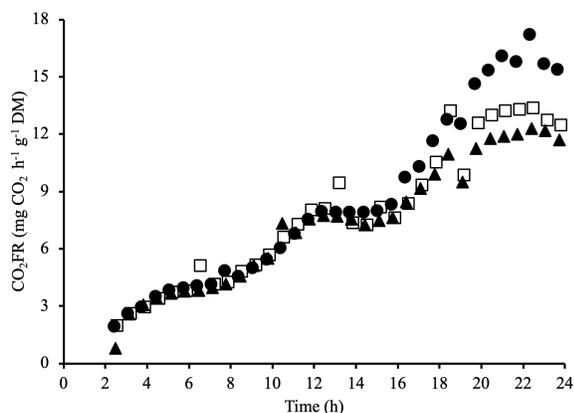


Fig. 2. CO<sub>2</sub> formation rate (CO<sub>2</sub> FR, mg CO<sub>2</sub> g<sup>-1</sup> DM h<sup>-1</sup>) evolution during aerobic pretreatment of the OSW for the three temperature conditions assayed. a) 30°C isothermal (●); b) 12 h at 30°C and 12 h at 35 °C (▲) and c) 12 h at 35 °C and 12 h at 40°C (□).

The CO<sub>2</sub> formation rates showed a similar behaviour independently of temperature treatment reaching different values of CO<sub>2</sub> production. In the case of the isothermal test at 30 °C, the maximum rate of CO<sub>2</sub> formation (17.2 mg CO<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> DM) was observed at 22 h of incubation, with a pH increase of 6.2 to 7.4. In the second pretreatment (12 h at 30 °C and 12 h at 35 °C), the maximum rate of CO<sub>2</sub> formation was 12.3 mg CO<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> DM and was observed at 22 h of incubation, showing a pH increase of 6.2 to 7.4. With the third incubation condition (12 h at 35 °C and 12 h at 40 °C), the maximum rate was 13.4 mg CO<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> DM at 22.5 hours, with a slight increase in pH (6.6). In the three pretreatments, pH increase could be due to the formation of carbonates, as result of the high solubility of CO<sub>2</sub> in the wet fraction of the OSW mixture, as reported by Dahiya *et al.* (2015). The results show significant differences between the rate of formation of CO<sub>2</sub> of the first condition of temperature (30 °C), with respect to the second and third conditions with a level of significance of  $\alpha = 0.05$ . These indicates that a higher rate of CO<sub>2</sub> formation can be obtained, under isothermal temperature conditions, according to what was reported by Ekinici *et al.* (2004).

### 3.2 Enzymatic activity

Figure 3 (a, b, c, d, and e) shows the profile of the enzymatic activities (amylase, cellulase, pectinase, xylanase and protease (U g<sup>-1</sup> DM) during the aerobic pretreatment of OSW for 24 hours at the three incubation conditions tested.

The maximum activity of the enzyme amylase (60 U g<sup>-1</sup> DM) occurs with the third pretreatment (35-40 °C) after 6 hours of incubation. Some authors have reported that the activity of amylase in the early degradation of organic compounds can be attributed to the increase in microbial biomass during the start of composting (Castaldi *et al.*, 2008). In this work, the activity of the amylase obtained is similar to reported by Castro *et al.* (2015) after 120 hours of incubation at 40 °C, and below the value reported by Hashemi *et al.* (2011) after 60 hours of incubation at 37 °C. In this work, no heat treatment was applied to the residues, so the activity of this enzyme was induced on the crude starch.

The cellulase activity shows its maximum activity (69 U g<sup>-1</sup> DM) with the second pretreatments (30-35 °C) and third (35-40 °C) for 6 and 18 hours of incubation respectively.

Table 1. Characterization of OSW before and after aerobic pretreatment.

Parameters	Units	OSW without pretreatment	OSW with aerobic pretreatment		
			OSW TBR <sub>1</sub> (30 °C)	OSW TBR <sub>2</sub> (30-35 °C)	OSW TBR <sub>3</sub> (35-40 °C)
Initial Mass	g wet basis	-	150	150	150
Total Solids	gkg <sup>-1</sup> wet basis	233 ± 2 <sup>b</sup>	207 ± 3 <sup>a</sup>	209 ± 2 <sup>a</sup>	208 ± 5 <sup>a</sup>
Moisture	%	76.7	79.3	79.1	79.2
Volatile solids	gkg <sup>-1</sup> dry basis	789 ± 24 <sup>b</sup>	845 ± 33 <sup>a</sup>	851 ± 29 <sup>a</sup>	841 ± 26 <sup>a</sup>
Total carbohydrates	gkg <sup>-1</sup> dry basis	184 ± 14 <sup>a</sup>	173 ± 17 <sup>a</sup>	177 ± 19 <sup>a</sup>	173 ± 23 <sup>a</sup>
Initial pH		6.25	6.25	6.25	6.25
Final pH		-	7.4	7.4	6.6
Density	gcm <sup>-3</sup>	0.78 ± 0.02 <sup>b</sup>	0.89 ± 0.07 <sup>a</sup>	0.88 ± 0.03 <sup>a</sup>	0.89 ± 0.05 <sup>a</sup>

Same letter by column means no significant difference (Tukey HDS,  $\alpha = 0.05$ ) between samples.

According to the literature, the activity of cellulase depends on the type of microorganisms that develop in organic waste (Goyal *et al.*, 2005). This is because fungi are mainly involved in the decomposition of cellulose, hemicellulose, and lignin, and the growth of this type of microorganisms in the residues could have favored the synthesis and activity of these enzymes (Goyal *et al.*, 2005).

The maximum activity of cellulase detected in this work is two times higher than the value reported by Leite *et al.* (2016) over 11 days of fermentation, and 10 times greater than that reported by Castro *et al.* (2015) in 120 hours of seed waste composting.

In the case of pectinase, its maximum activity (74 U g<sup>-1</sup> DM) is shown under isothermal conditions (30 °C), after 18 hours of incubation. The value of the maximum pectinase activity in this work is double to that reported by other authors (Botella *et al.*, 2007) using grape residues and 12 times higher than that reported by Romo *et al.* (2014) using agroindustrial waste.

The xylanase showed its highest activity at 18 hours for the isothermal pretreatment with a maximum value of 111 U g<sup>-1</sup> DM. The maximum activity of xylanase determined in this work was higher than that obtained by Leite *et al.* (2016) and 2.7 times higher to that reported by other authors for the composting of urban solid waste (Castaldi *et al.*, 2008; Raut *et al.*, 2008). In this work, a greater activity of the xylanases was obtained with respect to the rest of the other quantified enzymes, this is important since this enzyme contributes to the breakdown of the xylan molecules present in the hemicellulose polymers (Devia, 2014). This maximum activity was obtained when the pH of the medium was between 6-7.5, as reported by Martínez-Valdez

*et al.* (2015); Castañeda-Casasola *et al.* (2018). Rodríguez-Fernandez *et al.* (2011) reported that the type and quantity of microorganisms present in the environment, as well as the heterogeneous nature of the waste, favours the activity of these enzymes. The proteases presented a different behaviour compared to other hydrolytic enzymes. In this work, maximum protease activity was detected at the start of the aerobic pretreatment in the three conditions assayed (101 U g<sup>-1</sup> DM), subsequently, the activity gradually decreased. Some authors have reported that protease activity may occur initially during the composting process, due to the availability of polypeptides in the waste over a wide range of temperature (15-45 °C), as well as during the removal of ammonia through aeration, because the accumulated ammonia could act as an inhibitor during the process (Mamma *et al.*, 2008). It has also been reported that the activities of proteases depend strongly on the nature of the waste and the conditions of the bioprocesses (Castaldi *et al.*, 2008). The value obtained in this work is four times higher than those reported by other authors in the aerobic degradation of organic waste (Martínez-Valdez *et al.*, 2016).

### 3.3 Characterisation of OSW before and after aerobic pre-treatment

Table 1 shows the results of the characterisation of the OSW mixture before and after the aerobic pre-treatment for 24 hours. The aerobic pre-treatment produced a reduction of up to 6% of total carbohydrates contained in the OSW, due to the consumption of compounds that are rapidly metabolised by the microbial population, such as sugars (Sundberg *et al.*, 2004).

Table 2. Characterization of OSW after hydrolysis and acidogenesis.

Parameters	Units	OSW AHLBc	OSW AHLB <sub>1</sub>	OSW AHLB <sub>2</sub>	OSW AHLB <sub>3</sub>
Total Solids	gkg <sup>-1</sup> wet basis	218 ± 9 <sup>b</sup>	148 ± 5 <sup>a</sup>	153 ± 5 <sup>a</sup>	149 ± 7 <sup>a</sup>
Moisture	%	78.2	85.2	84.7	85.1
Volatile solids	gkg <sup>-1</sup> dry basis	834 ± 26 <sup>c</sup>	513 ± 18 <sup>a</sup>	529 ± 22 <sup>b</sup>	523 ± 26 <sup>b</sup>
COD <sub>Total</sub>	gkg <sup>-1</sup> dry basis	871 ± 27 <sup>b</sup>	522 ± 21 <sup>a</sup>	536 ± 27 <sup>a</sup>	543 ± 23 <sup>a</sup>
Total Carbohydrates	gkg <sup>-1</sup> dry basis	68 ± 4 <sup>b</sup>	20 ± 3 <sup>a</sup>	26 ± 4 <sup>a</sup>	27 ± 3 <sup>a</sup>
Final pH		4.77	6.1	5.03	5.27
Density	gcm <sup>-3</sup>	0.78 ± 0.07 <sup>b</sup>	0.89 ± 0.08 <sup>a</sup>	0.88 ± 0.04 <sup>a</sup>	0.89 ± 0.07 <sup>a</sup>
Final matter	g wet basis	112.1	119.2	137.13	138.85
Final matter	g dry basis	24.43	17.64	20.98	20.69

Same letter by column means no significant difference (Tukey HDS,  $\alpha = 0.05$ ) between samples.

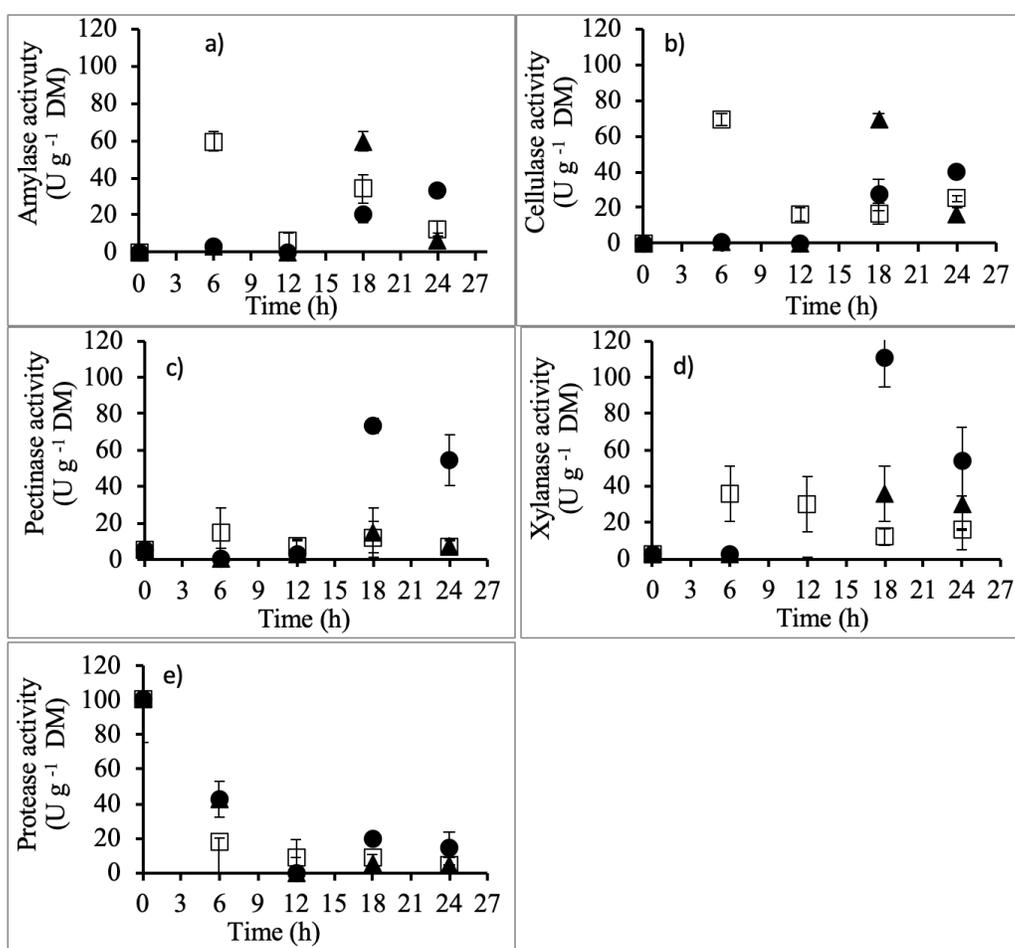


Fig. 3. Enzymatic activities during aerobic pretreatment of OSW incubation for 24 hours. a) amylase; b) cellulase; c) pectinase; d) xylanase and e) protease. Symbols represent different temperatures 30°C (●), 30-35°C (▲) and 35-40°C (□). Values are means of two replicates ± standard deviation.

However, volatile solids increased 7%, probably due to the increase in biomass, which is related to CO<sub>2</sub> production. This is because it is an indicator of microbial activity which is related to enzymatic activity. The microbial activity is achieved through the action of enzymes that are responsible for the hydrolysis of complex macromolecules that constitute organic waste (Vargas-García *et al.*, 2010). A pH increase was observed up to a value of 7.4; it could be related to the metabolism of aerobic microorganisms and the high solubility of CO<sub>2</sub> in the wet fraction of the mixture of the OSW inside the TBR's (Dahiya *et al.*, 2015).

### 3.4 Hydrolysis and acidogenesis of the OSW after aerobic pretreatment

Table 2 shows the results of the characterisation of the OSW mixture after hydrolysis and acidogenesis for 21 days in the 4 AHLB reactors. In Table 2, are presented the best results obtained in the AHLB<sub>1</sub> reactor. A reduction of total solids, volatile, COD and carbohydrates of 13, 16, 15 and 17% respectively with respect to the AHLBc reactor and a higher value of pH 6 at the end of the fermentation. According to other authors favours the hydrolysis of the compounds present in the OSW (Kim *et al.*, 2003). Therefore, the aerobic pretreatment showed a positive effect on the hydrolysis and acidogenesis of the OSW mixture fed to the 4 AHLB reactors, possibly due to the activity of exoenzymes generated during the aerobic pretreatment (Figure 3). These results are similar to those reported by Xu *et al.* (2014), who applied a short aerobic pretreatment (2-6 hours) at 35°C on solid food waste before carrying out a two-stage anaerobic digestion treatment.

### 3.5 Evolution of pH, COD, and VFA in the leachates of AHLB reactors

Figure 4 (a, b and c) shows the pH profile, the COD concentration and the VFA concentration in the leachates produced in the 4 AHLB reactors. A significant decrease in pH was observed on the first day of the anaerobic treatment, due to the fermentation processes. These results coincide with those reported in the literature since the hydrolysis of high molecular weight compounds promotes a progressive increase in the concentration of soluble compounds measured as COD and VFA in the leachates (Ramírez *et al.*, 2014; Rodríguez-Pimentel *et al.*, 2015). For the AHLB<sub>1</sub> and AHLB<sub>2</sub> reactors, the pH decreased to 5.5 and

remained at that value until day 18. Unlike the AHLB reactors fed with aerobic pre-treated wastes, the pH of the control reactor (AHLBc) decreased to 4.5 and remained constant at that value until day 21. The pH increase in the pre-treated AHLB<sub>3</sub> could occur by the formation of carbonates during aerobic pre-treatment, due to the high solubility of CO<sub>2</sub> (Dahiya *et al.*, 2015).

Figure 4b shows the concentration of soluble organic matter in the leachates of the four AHLB reactors. A significant increase of 77gL<sup>-1</sup> of soluble COD on day 11 of fermentation in the AHLB<sub>1</sub> reactor is observed with the aerobic pretreatment at 30 °C, this COD concentration is two times higher compared with AHLBc reactor (41gL<sup>-1</sup>).

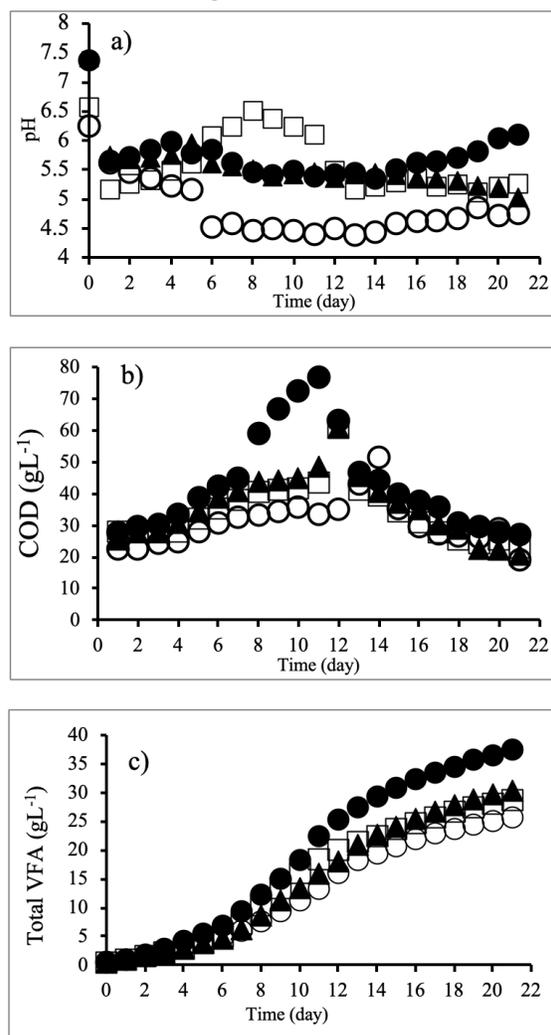


Fig. 4. Profile of pH (a) and the chemical oxygen demand (b) of the leachates analyzed in the four AHLB reactors. Total VFA in the four anaerobic reactors: AHLBc (○), AHLB<sub>1</sub> (●), AHLB<sub>2</sub> (▲), AHLB<sub>3</sub> (□).

Aerobic pretreatment under these conditions markedly improved the hydrolysis of the biopolymers present in the OSW and their formation insoluble compounds that were carried in the leachate (Kim *et al.*, 2003). The AHLB<sub>2</sub> and AHLB<sub>3</sub> reactors had a COD concentration of 60 gL<sup>-1</sup> on day 12 of fermentation. The profile of the soluble COD showed a behaviour similar to that reported by Ramírez *et al.* (2014) and Rodríguez-Pimentel *et al.* (2015) who worked with different compositions of the organic fraction of municipal solid waste (OFMSW) using the same reactor configuration as in this work. After day 18, degradation of organic matter occurs slowly, indicating the stabilisation of biosolids. The time required for the stabilisation of these residues was less than that reported by Ramirez *et al.* (2014) and Rodríguez-Pimentel *et al.* (2015), who reported a time of stabilization of the COD of 38 and 27 days, respectively, which favoured the hydrolysis rate. Some authors report that the addition of mature compost to OFSW improves the solubilisation of organic matter during anaerobic digestion (Xu *et al.*, 2014).

Figure 4c shows the profile of the concentration of volatile fatty acids (VFA) in the leachate of the AHLB reactors; the AHLB<sub>1</sub> reactor presented the highest concentration (37gL<sup>-1</sup>) at day 21 of fermentation. The AHLB<sub>2</sub> and AHLB<sub>3</sub> reactors have similar concentrations (29 and 30gL<sup>-1</sup>). The experimental evidence shows a 48% increase in the VFA concentration of the aerobic pre-treatment at 30 °C compared to the AHLB reactor without pre-treatment, suggesting that the aerobic pre-treatment also favoured an increase in acidogenesis. Acetic acid was the most abundant compound and represented approximately 70% of the total VFA in the four AHLB reactors. The production of acetic acid in the four AHLB reactors increased rapidly and its concentration in the leachate reached values of 30gL<sup>-1</sup> in the first 14 days of operation. In addition to acetic and butyric acids, the production of propionic and valeric acid was

also observed, at a lower concentration. These results are consistent with reports of observation that micro-aeration treatments enhance the anaerobic hydrolysis of municipal solid waste and food residues (Xu *et al.*, 2014). In this work, the increase in the concentration of acetic and butyric acid was observed in parallel with the pH decrease within each of the AHLB reactors, where the pH value was observed in a range between 4.8 to 6.1. According to other authors, this pH range favours the fermentation of rapidly degradable organic compounds during the hydrolysis-acidogenesis phase, while the hardly degradable organic compounds favour the production of propionic acid (Xu *et al.*, 2014).

### 3.6 Efficiencies of the anaerobic process

From the results of Table 2, the removal efficiencies of the volatile solids, COD and total sugars were calculated, as well as the hydrolysis constant (*k*) and the rate of formation of the VFA in the leachates, Table 3 shows these results.

In Table 3, it can be seen that the best results were obtained in the AHLB<sub>1</sub> reactor, and since the highest elimination efficiency was reached for COD, VS, and carbohydrates, these values were 32%; 33% and 87%, respectively. The productivity of VFA presented a maximum value in the AHLB<sub>1</sub> reactor with a value of 0.82 g L<sup>-1</sup> d<sup>-1</sup>, which was 2.5 times higher concerning the rate of production of VFA in the AHLB control reactor. The hydrolysis constants (*k*) showed values between 0.06 and 0.08 d<sup>-1</sup>, values that are 3 times higher than those reported by Ramírez *et al.* (2014). Also these values are until 2 times greater than those reported by Rodríguez-Pimentel *et al.* (2015), who carried out the anaerobic digestion in two-stage of the OFMSW, using the same reactor configuration (AHLB), with temperature at 30 ± 2 °C and without applying any aerobic treatment prior to the anaerobic digestion of the waste (Ramírez *et al.*, 2014; Rodríguez-Pimentel *et al.*, 2015).

Table 3. Anaerobic hydrolytic leach bed (AHLB) reactors performance indicators.

Reactor	Initial pH	Final pH	<i>k</i> (d <sup>-1</sup> )	$\eta_{\text{COD}}$ (%)	$\eta_{\text{VS}}$ (%)	$\eta_{\text{CHO}}$ (%)	$r_{\text{VFA}}$ (g L <sup>-1</sup> d <sup>-1</sup> )
AHLBc	6.25	4.77	0.06	15 ± 2.1 <sup>a</sup>	15 ± 1.3 <sup>a</sup>	71 ± 5.1 <sup>a</sup>	0.32 ± 0.02 <sup>b</sup>
AHLB <sub>1</sub>	7.37	<b>6.1</b>	<b>0.081</b>	32 ± 2.4 <sup>c</sup>	33 ± 3.2 <sup>c</sup>	87 ± 4.5 <sup>bs</sup>	0.82 ± 0.04 <sup>c</sup>
AHLB <sub>2</sub>	7.42	5.03	0.067	23 ± 2.1 <sup>b</sup>	22 ± 1.8 <sup>b</sup>	81 ± 3.3 <sup>b</sup>	0.32 ± 0.02 <sup>b</sup>
AHLB <sub>3</sub>	6.57	5.27	0.062	18 ± 1.3 <sup>b</sup>	20 ± 1.6 <sup>b</sup>	80 ± 2.9 <sup>b</sup>	0.19 ± 0.01 <sup>a</sup>

*k*: hydrolysis constant;  $\eta$ : degradation efficiency; COD: chemical oxygen demand; VS: volatile solids; CHO: carbohydrates; *r*: VFA production rate.

Same letter by column means no significant difference (Tukey HSD,  $\alpha = 0.05$ ) between samples.

However, Bolzonella *et al.* (2005) using fully stirred reactors, reported hydrolysis constants with a higher value ( $0.11 \text{ d}^{-1}$ ) than that obtained in this work. It indicates that the operation conditions of the reactor has an important influence on the hydrolysis of organic compounds present in urban solid waste during the hydrolysis process and acidogenesis, although with an energetic increase using agitation. In this context, the literature reports that a strong acidic pH negatively affects the value of the hydrolysis constant, but a pH between 4 and 6 favours acidogenesis (Kim *et al.*, 2003).

Therefore, the results with 24-hour aerobic pre-treatment at  $30 \text{ }^\circ\text{C}$  are in agreement with that reported in the literature, where low enzyme activities, basic pH and a low  $\text{CO}_2$  formation rate indicate that the organic matter is in the process of stabilisation and microbial activity gradually ceases (Beck-Friis *et al.*, 2001; Tejada *et al.*, 2009). This pre-treatment was the factor that showed a greater significant effect on the solubilisation performance, on the organic solid residues. According to the statistical analysis, the hydrolysis and acidogenesis resulted in the higher values when the organic matter was expressed in terms of COD, VFA and VS, which is in accordance with what was reported by Charles *et al.* (2009) and Xu *et al.* (2014).

## Conclusions

Aerobic pretreatment for 24 hours at  $30 \text{ }^\circ\text{C}$ , showed a significant effect on  $\text{CO}_2$  production ( $182 \text{ mg de CO}_2 \text{ g}^{-1} \text{ DM}$ ), with respect to the two remaining incubation conditions during aerobic pre-treatment and on the results obtained after the fermentation in the control reactor without pre-treatment. Similar behaviour was viewed in results of the enzymatic activities (xylanases and pectinases) and in the hydrolysis and acidogenesis of the organic solid wastes with a hydrolysis constant ( $k$ ) of  $0.081 \text{ d}^{-1}$  and a productivity of VFA of  $0.82 \text{ g L}^{-1} \text{ d}^{-1}$ . This experimental evidence shows the potential of using a short aerobic pre-treatment, to improve the production of hydrolytic enzymes and favour the acidogenesis of the OSW in AHLB reactors, as an alternative for obtaining high concentrations of VFA in the leachates and to increase methane productivity in high-rate anaerobic reactors like the UASB.

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## Nomenclature

AHLB	Anaerobic hydrolytic leach bed
$\text{CO}_2\text{FR}$	$\text{CO}_2$ formation rate
COD	Chemical oxygen demand
F	Leachate flow ( $\text{L d}^{-1}$ )
FID	Flame ionization detector
FS	Fixed solids
DM	dry matter
$k$	Hydrolysis constant ( $\text{d}^{-1}$ )
NMX	Norma mexicana
OSW	Organic solid wastes
HALE	HidrolíticoAnaerobio de Lecho Ecurrido
RSO	Residuos Sólidos Orgánicos
$S_0$	Initial concentration ( $\text{g COD L}^{-1}$ )
$S_1$	Final concentration ( $\text{g COD L}^{-1}$ )
SRT	Solids retention time
$t$	tiempo, d
$S_{y0}$	Initial concentration ( $\text{g VS L}^{-1}$ )
$S_{y1}$	Final concentration ( $\text{g VS L}^{-1}$ )
$S_y$	Concentration ( $\text{g VS g}^{-1} \text{OSW}$ )
TBRs	Tubular reactors
TBR <sub>1</sub>	Isothermal at $30^\circ\text{C}$ for 24 h
TBR <sub>2</sub>	Reactor incubated at $30^\circ\text{C}$ for 12 h, and then incubated at $35^\circ\text{C}$ for another 12 h
TBR <sub>3</sub>	Reactor incubated at $35^\circ\text{C}$ for 12 h, and then incubated at $40^\circ\text{C}$ for another 12 h
TCD	Thermal conductivity detector
TCDF	Total $\text{CO}_2$ formation
TS	Total Solid
UASB	Upflow anaerobic sludge blanket
V	Reactor volume (L)
VL	Volume of leachate (L)
VFA	Volatile fatty acids
VS	Volatile solid
W <sub>0</sub>	Initial mass ( $\text{g OSW d}^{-1}$ )
W <sub>1</sub>	Final mass ( $\text{g OSW d}^{-1}$ )
$^\circ\text{C}$	degrees Celsius
AGV	Ácidos Grasos Volátiles
MS	Materia Seca
OFMSW	organic fraction of municipal solid waste
MAU	Metropolitan Autonomous University
$\text{CH}_4$	methane

CO <sub>2</sub>	carbon dioxide
T <sub>1</sub>	30°C
T <sub>2</sub>	30°C for 12 h, and then incubated at 35°C for another 12 h
T <sub>3</sub>	35°C for 12 h, and then incubated at 40°C for another 12 h
C/N	carbon/nitrogen ratio
N <sub>2</sub>	Nitrogen
vkgm	volumetric airflow per kilogram of mixture
<i>Greek symbols</i>	
$\alpha$	level of significance
$\nu$	degradation efficiency
$r$	production rate

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