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Influence of composition on the biomethanation potential of restaurant waste at mesophilic temperatures

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Abstract

A synthetic waste was used to study the effect of waste composition on anaerobic degradation of restaurant waste. It was made by blending melted pork lard, white cabbage, chicken breast, and potato flakes, to simulate lipids, cellulose, protein, and carbohydrates, respectively. Four blends of the four constituents with an excess of each component were assayed and compared with a fifth blend containing an equal amount of chemical oxygen demand (COD) of each of the four components. The methane production and the time course of soluble COD and volatile fatty acids were assessed in batch assays. A high reduction of volatile solids (between 94% and 99.6%) was obtained in all the assays. The methane yield was between 0.40 m^3 CH_4/kg $VS_{initial}$ (excess of carbohydrates) and 0.49 m^3 CH_4/kg $VS_{initial}$ (excess of lipids). The degradation of the lipid-rich assays differed from the others. Fifty percent of the biochemical methane potential was obtained after 3–6 days for all of the assays, except for the one with excess of lipids which achieved 50% methanation only after 14.7 days of incubation. In the assay with excess of lipids, a considerable fraction of COD remained in the liquid phase, suggesting an inhibition of the methanogenic process that was likely due to the accumulation of long chain fatty acids. The hydrolysis rate constants, assuming first order kinetics, over the first 6 days were between 0.12 d^{-1} (excess of lipids) and 0.32 d^{-1} (excess of carbohydrates). The results indicate that anaerobic digestion facilities with large variations in lipid input could have significant changes in process performance that merit further examination.

1. Introduction

Although anaerobic digestion of organic solid wastes is an established technology in Europe with 120 full scale plants treating about 4 million tons per year, it represents, on average, only 27.5% of all of the biological waste treatment processes (De Baere, 2006).

Kitchen waste is a large fraction of municipal solid waste (20–65%) (Tchobanoglous et al., 1993). The biomethanation potential of the waste depends on the relative amounts of the four main components – proteins, lipids, carbohydrates, and cellulose. Kitchen and restaurant waste are not homogeneous in day-to-day composition. It is important to have data to predict how these fluctuations may influence the anaerobic digestion process.

This work aims to study how variations of the major components of restaurant waste influence the methane yield and the process kinetics.

2. Materials and methods

2.1. Waste characterization

A synthetic restaurant waste, representing the major components of waste from a real restaurant prepared by mixing melted lard of pork, white cabbage, chicken breast, and potato flakes, to simulate lipids, cellulose, protein and carbohydrates, respectively. A preliminary assay was done in order to assess the adequacy of the synthetic waste to simulate a real restaurant waste. The restaurant waste

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was a composite sample (1 week based) from the waste produced in the restaurant of the University of Minho, located in "Campus de Gualtar", Braga, Portugal. The characteristics of each component of the synthetic waste and of the real waste are presented in Table 1. The particle size was in the range of 1–3 mm. The different mixtures were prepared immediately before launching the tests. The real restaurant waste was collected, ground to 1–3 mm particle size and stored at 4 °C during 5 days, until the end of the collection process. Then it was mixed and stored at −18 °C.

2.2. Inoculum

The granular sludge used as inoculum was collected from an upflow anaerobic sludge blanket reactor treating a brewery effluent located in Oporto, Portugal. The solid content of the inoculum was 100 mgVS/g of sludge. The VS/TS was 65%. The use of a granular sludge as an inoculum had been tested previously and shown to reduce the risk of over-acidification during batch, high-solids, anaerobic digestion (Neves et al., 2004). Based on these previous studies, the use of the granular sludge as an inoculum appears to lead to rapid digestion of food waste without the need for a lengthy acclimitization period.

The production of methane due to the residual substrate present in the inoculum was recorded in a parallel blank assay where only the inoculum was incubated without any substrate. The values obtained in this experiment were used to correct the cumulative methane production values in all the assays with the exception of the preliminary assay comparing the real and the synthetic waste. The inoculum was also characterised in terms of the Specific Methanogenic Activity in the presence of acetate and H_2/CO_2, the two trophic groups directly involved in methane production. The obtained values were 0.92 ± 0.20 and 2.96 ± 0.03 g COD-CH_4/gVS d, respectively.

The quantification of the residual methane production was performed by measuring the methane production in sealed vials without substrate. A pressure transducer was used to record the increase in pressure, and headspace biogas was sampled periodically to assess the methane content. Strict anaerobic conditions were maintained by using an anaerobic basal medium composed of cysteine HCL (0.5 g/L), $NaHCO_3$ (3 g/L), with the pH adjusted to 7.0–7.2. Resazurin was added as an indicator of redox potential. This basal medium was prepared by boiling the medium before adding the bicarbonate. The handheld pressure transducer was capable of measuring a pressure increase or decrease of two atmospheres (0 to ±202.6 kPa) over a range of −200 to +200 mV. The sensing element is connected to a digital panel module and the device is powered by a 9.0 V DC transformer. The same technique was used to assess the specific methanogenic activity, but individual substrates (acetate – 30 mM and H_2/CO_2 80:20 V/V at 1 Bar overpressure) were added to the vials. All the batch assays described were performed in triplicate assays. The volume of methane produced was corrected to Standard Temperature and Pressure conditions (STP – 1 atm and 273 K).

2.3. Batch experiments

Two preliminary batch experiments were done in order to verify the suitability of the synthetic waste to represent a real restaurant waste. Methane production was followed from the real restaurant waste and from the synthetic restaurant waste after incubation in batch vials in the following conditions: 5% total solids (TS), 1.35 g volatile solids $(VS)_{waste}/gVS_{inoculum}$. The synthetic waste was made by blending the different components in equal amount of TS (125 mg). After introducing the correct amounts of waste and seed sludge, a defined amount of anaerobic basal medium (described above) was added under strict anaerobic conditions, in order to give the desired solids content. The vials were then incubated at 37 °C under stirring conditions (150 rpm) and the pressure increase was recorded using the above mentioned pressure transducer device. The biogas accumulated in the headspace was sampled regularly and the methane content was determined. Pressure and headspace methane content data were used to calculate the volume of methane produced and then corrected to STP conditions. The results were expressed in terms of methane yield (m^3 $CH_4/kgVS_{initial}$) and % of methanation that corresponds to the % of methane produced relative to the biochemical methane potential (0.350 m^3 CH_4 $_{(STP)}$ /kg COD from stoichiometry).

Table 1
Characterization of the synthetic and the real restaurant waste

Waste	Individual components of the synthetic restaurant waste				Real restaurant waste
	Fat (lard)	Cabbage	Chicken breast	Potato flakes	
COD (mg/g_{ww}[a])	1632 ± 38	53 ± 7	306 ± 70	1018 ± 106	327 ± 73
TS (mg/g_{ww}[a])	970 ± 31	58 ± 1	330 ± 13	930 ± 14	238 ± 1
VS (mg/g_{ww}[a])	974 ± 30	56 ± 1	320 ± 28	893 ± 3 1	214 ± 7.0
TKN (mg $N–NH_4/g_{ww}$[a])	0.57 ± 0.10	Not detected	52.3 ± 3.6	9.3 ± 1.0	13 ± 1
Fat content (mg/g_{ww}[a])	977 ± 18	Not detected	8.5 ± 1.9	16.3 ± 1.2	20 ± 8
Moisture content (%)	3 ± 3	94.2 ± 0.1	67.0 ± 1.3	7.0 ± 1.4	76.2 ± 0.1

TKN, Total Kjeldahl Nitrogen.
Values given are averages and standard deviations based on 25 measurements.
[a] g_{ww}, mass of raw waste express in grams of wet weight.

After this preliminary experiment, two sets of assays were performed in order to study the influence of the excess of each component present in the synthetic waste in terms of biogas production and process kinetics. In the first set of batch assays, the biogas production and composition was assessed as previously described. In the second set of batch tests, the liquid composition was assessed in terms of VFA and soluble COD. Table 2 presents the experimental conditions prevailing in both assays.

The liquid composition assays were performed in 600 mL flasks, keeping all of the ratios and conditions applied in the second set of the methanation assays. Liquid samples were regularly withdrawn, centrifuged, and filtered (0.2 μm pore size membranes) for soluble COD and VFA (acetate, propionate, iso-butyrate and *n*-butyrate) analysis. These batch tests were performed in duplicate assays.

Table 2
Experimental conditions prevailing in the batch assays

	Waste component	Gas production assays	Liquid composition assays
% Total solids		1.8	1.8
Waste/inoculum (gVS/gVS)		1.35	1.35
Total volume (mL)		160	600
Anaerobic medium added (mL)		10	200
Initial COD (g/L)		16	16
Excess lipids (mg COD added)	Fat (lard)	70	1400
	Cabbage	30	600
	Chicken breast	30	600
	Potato flakes	30	600
Excess cellulose (mg COD added)	Fat (lard)	30	600
	Cabbage	70	1400
	Chicken breast	30	600
	Potato flakes	30	600
Excess protein (mg COD added)	Fat (lard)	30	600
	Cabbage	30	600
	Chicken breast	70	1400
	Potato flakes	30	600
Excess carbohydrates (mg COD added)	Fat (lard)	30	600
	Cabbage	30	600
	Chicken breast	30	600
	Potato flakes	70	1400
Control (mg COD added)	Fat (lard)	40	800
	Cabbage	40	800
	Chicken breast	40	800
	Potato flakes	40	800

2.4. Analytical methods

COD, TS, VS and Total Kjeldhal Nitrogen (TKN) were determined according to Standard Methods (APHA et al., 1989). The closed reflux titration method was used for COD analysis. A defined amount of waste was previously suspended in hot water and homogenised with a Euroturrax T20 Standard (Ika Labortechnik) homogeneizer. For TKN analysis a defined amount of the solid waste was directly digested using selenium as catalyst. The fat content was extracted with a mixture of chloroform:methanol 1:2 (v:v) in a soxtec system, dried and weighed. The methane content of the biogas was measured by gas chromatography using a Porapack *Q* (180–100 mesh) column, with helium as the carrier gas at 30 mL/min and a thermal conductivity detector. Temperatures of the detector, injector and oven were 110, 110 and 35 °C, respectively. VFA were determined by high-performance liquid chromatography using a chrompack column (300 × 6.5 mm) and a mobile phase of sulphuric acid 5 mM at 0.7 mL/min. The column was set at 60 °C and the detection was by spectrophotometry at 220 nm.

3. Results and discussion

In the first experiment, the cumulative methane production obtained from the real restaurant waste was compared with the methane production obtained from the synthetic waste (Fig. 1). This experiment was planned to indicate the adequacy of the synthetic waste to represent the real waste. Only the initial cumulative methane production was considered, which in eight days reached 56% of the theoretical methane yield for both wastes (196 mL CH_4/$gCOD_{added}$).

The similar initial methane production pattern obtained for the two wastes indicates that the synthetic waste was suitable to represent the real one. The advantage of using the synthetic waste in this study is that it allows the concen-

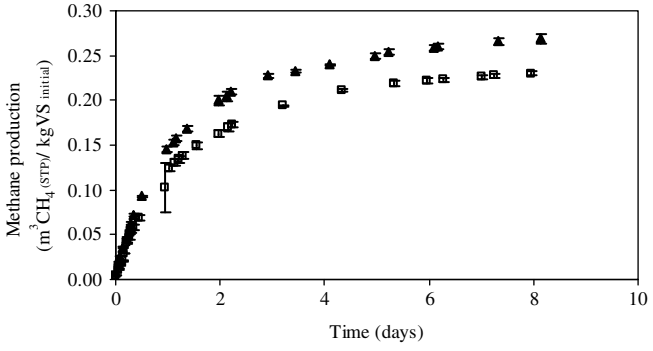

Fig. 1. Time course of the initial methane production obtained from the synthetic waste (□) and real restaurant waste (▲) when incubated in the following conditions: 5% total solids (TS), 1.35 g VS_{waste}/$gVS_{inoculum}$.The synthetic waste was made by blending the different components in equal amount of TS (125 mg). Values represent the average of duplicate experiments and *y*-bars represent the standard deviation.

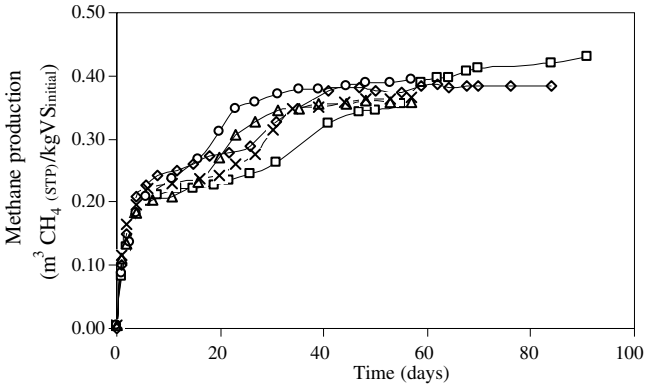

Fig. 2. Time course of cumulative methane production versus time in the assays with excess of lipids (□), cellulose (△), protein (○), carbohydrates (×) and the assay of equal COD amounts (◇).

trations of the major components of a restaurant waste to be changed.

Fig. 2 presents the time course of the methane production in all the assays.

All of the curves of methane production had, in general, similar behavior and displayed two plateaus. The first plateau was attained by day 3.8 for the assay with an excess of cellulose and around day 5.8–5.9 for the other assays. On average $50 \pm 5\%$ of the total methane production was achieved at this time in all of the assays, with the single exception of the assay with an excess of lipids (50% methanation only after 14.8 days). Although a different solids content was used in the preliminary experiment, a similar result was obtained where 50% of the theoretical methane potential was achieved after 4 days (Fig. 1). Therefore, it is expected that if the time period was extended in that experiment, a similar behavior would be observed.

Table 3 represents the time at which 50% and 85% of the theoretical methane potential were achieved. This % represents the obtained cumulative volume of methane divided per mass of COD added in relation to the biochemical methane potential of 0.35 m^3 CH_4/kg COD. The methane yield and the VS reduction are also presented. All of the values presented in Table 3 were corrected by subtracting the cumulative methane production obtained in the blank assays without waste.

The first assay to achieve 85% methanation was the one containing an excess of protein (after 23 days), followed by the assay containing an excess of cellulose (after 24 days), an excess of carbohydrates (in day 30), and an equal amount of COD (in day 32). The assay with an excess of lipids achieved 85% methanation only after 57 days.

The values of methane yield attained with the synthetic waste were in the range of values reported in the literature for the anaerobic digestion of the organic fraction of municipal solid waste. For instance, a methane yield of 0.301 m^3/kgSV was reported for the Valorga process (Valorga, 1985), while Mata-Alvarez (2003) reported a methane yield of 0.489 m^3/kgVS for the organic fraction of municipal solid waste for the city of Barcelona. The overall volatile solids reduction, including both inoculum and waste, was in the range of 94–99.6% (Table 3). The volatile solids change associated with the inoculum is not easy to assess because the inoculum contains substrates that are consumed and also are the source for biomass growth. In any case, the volatile solids change due to the inoculum is not as significant as for the waste.

The assays with an excess of cellulose, protein, carbohydrates, and equal COD presented maximum VFA concentrations between days 5 and 30, followed by a decrease and stabilization near a null value by day 50 (Fig. 3).

In general, the maximum soluble COD (not shown) and VFA concentrations occurred simultaneously with the first plateau observed in the cumulative methane production. The most significant VFA detected in the assays were acetic and *n*-butyric acids. The concentrations of VFA in assays containing an excess of protein were the highest and peaked at a value twice the amount detected in the other assays. It can be hypothesised that higher levels of proteins induced inhibition of methanogens by ammonia. In general, the concentration of VFA was approximately zero after 45 days of operation and corresponded to the stabilization in cumulative methane production. In the assay with lipids, a considerable amount of COD remained in the liquid phase as VFA after 80 days. This indicates that β-oxidation proceeded until the formation of butyrate, but further acetogenesis and methanogenesis were impaired.

The inhibition of methanogens in the assay with an excess of lipids can also be observed in Fig. 4 where the percentage of the acidified COD that was not converted into methane versus time for all of the assays is presented. Acidified COD was calculated as the sum of VFA-COD present in each liquid sample and the cumulative methane-COD production until the time of sampling.

Table 3
Time necessary to achieve 50% and 85% of the theoretical methane potential, final methane yield and VS reduction

Assay	Time for 50% methanation (days)	Time for 85% methanation (days)	Final methane yield (m^3 $CH_{4(STP)}$/kgVS initial)	Volatile solids reduction (%)
Excess lipids	14.8	57	0.43	96.5
Excess cellulose	3.9	24	0.36	95.1
Excess protein	5.9	23	0.39	94.0
Excess carbohydrates	3.0	30	0.37	99.6
Control	3.4	32	0.39	95.9

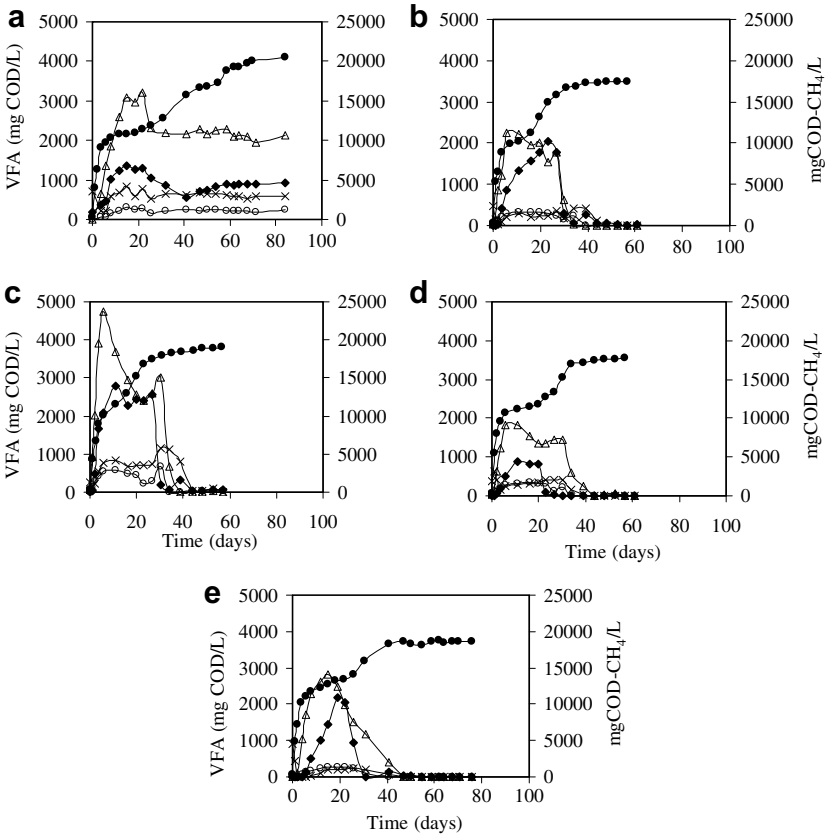

Fig. 3. Individual VFA concentrations (◆, acetic acid; ×, propionic acid; ○. i-butyric acid; △, *n*-butyric acid) and cumulative methane production (●), expressed as mg COD/L versus time in the assays with excess of lipids (a), cellulose (b), protein (c), carbohydrates (d) and the assay of equal COD amounts (e).

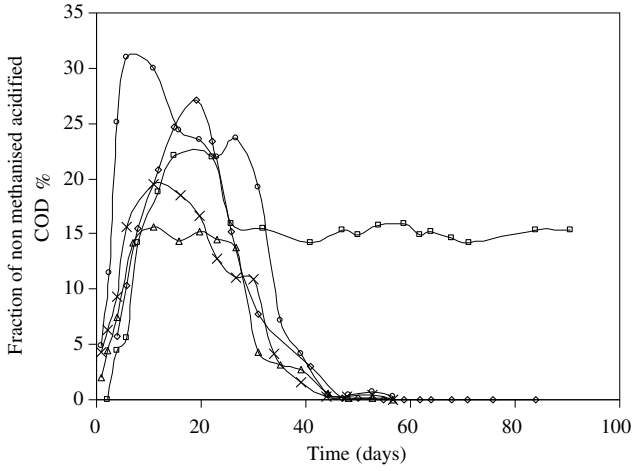

Fig. 4. Percentage of COD acidified but not methanised in the assays with an excess of lipids (□), cellulose (△), protein (○), carbohydrates (×) and the assay of equal amounts of COD (◇).

At the end of this assay, approximately 15% of the acidified COD that remained was not converted to methane. After hydrolysis of lipids, long chain fatty acids (LCFA) are produced, which are described in the literature as inhibitory to acidogenic and methanogenic populations (Rinzema, 1988; Angelidaki and Ahring, 1992). This was reported to be a reversible effect and can occur at a metabolic level or at a physical level. The reversibility of physical inhibition requires special conditions and was demonstrated in a study with oleic and palmitic acids (Pereira et al., 2005). It has been suggested that if the conversion of LCFA is slow, they can form a physical barrier that shields lipid surfaces from lipases, thus affecting the rate of hydrolysis (Rietsch et al., 1977; Verger, 1980). The production of biogas was also reported to affect the hydrolysis of lipids due to an emulsion effect. This effect decreases the size of the micelles and increases the available surface, thus increasing the hydrolysis rate constant (Sanders, 2001).

All of the assays except the lipid-rich ones exhibited relatively similar behaviour. In general, between days 5 and 30, there was a percentage of acidified COD that had not been converted into methane. This was more evident in the assay with an excess of protein, where approximately 30% of the acidified COD remained not methanised, most likely due to the presence of ammonium nitrogen that, depending on the pH, can inhibit the methanogenic population. However, after day 38, all of the acidified COD was converted into methane, indicating that the inhibitory problems were reversible.

It was possible to calculate the hydrolysis rate constants for each assay assuming a first order kinetics and following the procedure described by Sanders et al. (2003) (Eq. (1)) taking into account the values of initial particulate COD, soluble COD at different time intervals and the cumulative methane production. In the "classic" batch reactor approach followed, the degree of hydrolysis is calculated from the methane production and the production of soluble COD during digestion according to the following procedure:

$$-\frac{dX}{dt} = Kh \cdot X \quad (1)$$

where X is the COD concentration of the particulate substrate present in the assay at each time and Kh is the hydrolysis rate constant. The integration of this equation gives Eq. (2).

$$\ln\left(\frac{X}{X0}\right) = -Kh \cdot t \quad (2)$$

where X_0 is the COD concentration of the particulate substrate initially present in the vial. At each time, the COD concentration of the particulate substrate (X) was calculated according to Eq. (3):

$$X = X_0 - \sum_{i=1}^{n} \text{Hydrolised COD} \quad (3)$$

where i represents a sample taken from the medium in the liquid composition assays, n is the number of samples taken until time t, and the cumulative hydrolysed COD ($\sum_{i=1}^{n}$Hydrolised COD) is the sum of the soluble COD present until time t and the COD that has already been converted into methane, according to the Eq. (4):

$$\sum_{i=1}^{n} \text{hydrolised COD} = (\text{soluble COD})_t + \int_{t=0}^{t} (\text{COD-CH4})dt \quad (4)$$

where $(\text{solubleCOD})_t$ is the soluble COD measured on time t and $\int_{t=0}^{t}(\text{COD-CH4})dt$ is the cumulative methane production until time t, expressed as COD. The values of X were then calculated based on the initial particulate COD introduced in the vials (X_0), on the soluble COD measured in each of the n samples taken along the liquid composition assays and from the cumulative methane production curves. Fig. 5 represents the linear plots of $\ln X$ versus initial time. The hydrolysis rate constants were obtained from the slope of each line.

Table 4 compares the obtained values with literature values obtained for similar substrates.

The hydrolysis rate constants were in the range of 0.12–0.32 d^{-1}. The assays with an excess of carbohydrates and protein presented higher hydrolysis rate constants when compared to the assay containing an equal amount

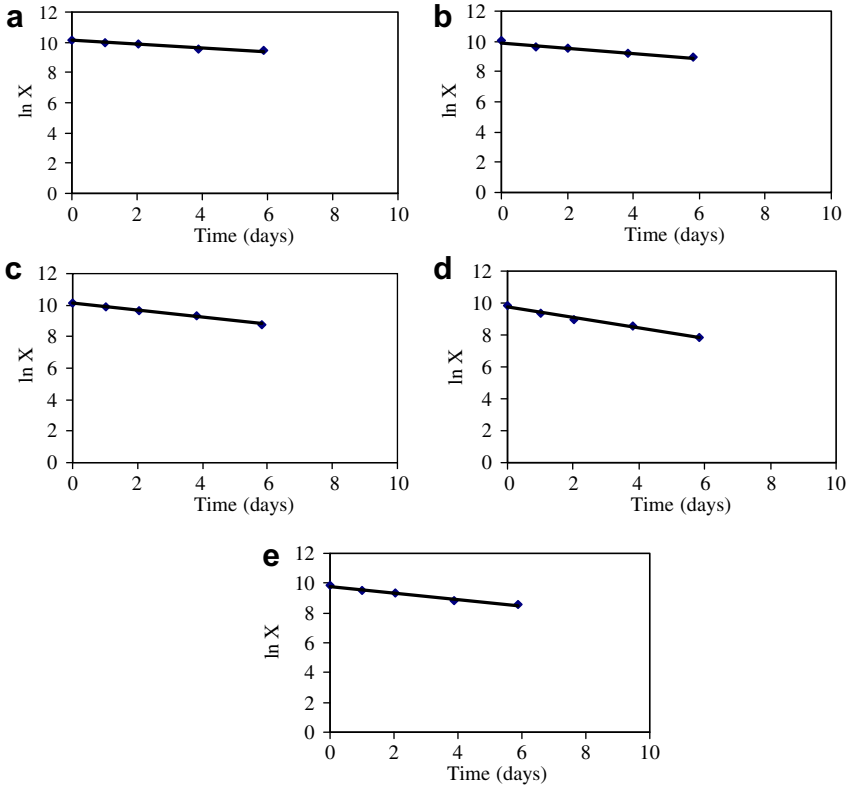

Fig. 5. Linear plot of ln(X) versus initial time in the assays with an excess of lipids (a), cellulose (b), protein (c), carbohydrates (d) and the assay of equal amounts of COD (e). The hydrolysis rate constants were obtained from the slope of each line.

Table 4
Values of the hydrolysis rate constants found in the tested batch assays over the first 6 days and values reported in the literature to similar substrates (assuming first order kinetics)

Reported literature	Substrate	Kh (d^{-1})	Temperature (°C)
Boon (1994)	Domestic sewer protein	0.2	35
García-Heras (2002)	Proteins	0.25–0.8	35
Present work	"Excess of protein"	0.24	37
García-Heras (2002)	Lipids	0.1–0.7	35
Present work	"Excess of lipids"	0.12	37
Greco et al. (1983)	Cellulose	0.12	35
Present work	"Excess of cellulose"	0.18	37
Boon (1994)	Starch	0.20–1.08	35
García-Heras (2002)	Carbohydrates	0.5–2	35
Present work	"Excess of carbohydrates"	0.32	37
Present work	"Equal COD amounts"	0.22	37

of COD for each component. The lowest values obtained for the hydrolysis rate constant were obtained in the assays with an excess of lipids and cellulose, indicating that when these components are in excess, a slower hydrolysis is induced. Lipids/LCFA can have a synergic effect on the degradation of all components present, since they adsorb onto solid surfaces and may delay the hydrolysis of other particulate compounds by reducing the accessibility of enzyme attack (Palenzuela Rollón, 1999). On the other hand, the possible adsorption of lipids/LCFA on the cells surface can hinder the access of simple substrates such as acetate, and therefore, methanogenesis can also be delayed.

4. Conclusions

Batch degradation of restaurant waste under methanogenic conditions depends on waste composition. If lipids are in excess a slower methane production, a higher concentration of COD in the liquid, and a lower hydrolysis rate constant is observed in comparison with a waste with equivalent amounts of COD of proteins, carbohydrates, lipids, and cellulose. One waste with an excess of carbohydrates and proteins presented hydrolysis rate constants higher (0.32 and 0.22 d^{-1}, respectively) than the wastes with an excess of lipids and cellulose (0.12 and 0.18 d^{-1}, respectively). The most efficient methane production rate and the lowest accumulation of volatile fatty acids were observed for the waste with an excess of carbohydrates. In this assay, after 3 days, 50% of the theoretical methane potential was achieved, although 30 days were needed to attain 85% biodegradability. The results found could be due to the choice of inoculum, and the use of anaerobic consortia that had been adapted to consuming varying loading rates of these organics might not show the same effects. The results point to the need for further work on digestion with other inocula. In addition, these results apply to particular wastes tested at one temperature, solids content, nutrient and toxin condition, and one should be careful before generalizing the findings to other test conditions. The present results indicate that anaerobic digestion facilities with large variations in lipid input could have significant changes in process performance that merit further examination.

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