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Influence of inoculum–substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: Process stability and kinetic evaluation

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ABSTRACT

A study of the anaerobic digestion of the solid waste generated in the extraction process of sunflower oil (sunflower oil cake, SuOC) was conducted at mesophilic temperature (35 °C) in batch mode. A laboratory-scale multi-reactor system was used to compare the volatile solids (VS) degradation and methane production (G) at inoculum–substrate ratios (ISRs) of 3.0, 2.0, 1.5, 1.0, 0.8 and 0.5 (expressed as VS basis). All tests were carried out against controls of inoculum without substrate. The stability and progress of the reaction from solid substrate to methane as an end product was monitored by measuring the pH, the soluble chemical oxygen demand, and the total volatile fatty acids-total alkalinity (TVFA/TA) ratio. The results obtained demonstrated that in the ISR range from 3.0 to 0.8, the pH ranged from 7.1 to 7.6 and this parameter was always stable during the anaerobic digestion process. In addition, within the above ISR range the TVFA/TA ratios were always lower than the failure limit values (0.3–0.4), which demonstrated the high stability of the anaerobic digestion process of this substrate at mesophilic temperature. Two kinetic models for substrate (VS) degradation and methane production were proposed and evaluated. The apparent kinetic constants for volatile solids degradation (K_1) and methane production (K_2) decreased from 0.54 ± 0.09 to $0.32 \pm 0.03\ d^{-1}$ and from 0.36 ± 0.04 to $0.16 \pm 0.03\ d^{-1}$, respectively, when the ISR decreased from 3.0 to 0.5, showing the occurrence of an inhibition phenomenon by substrate concentration. The kinetic equations obtained were used to simulate the anaerobic digestion process of SuOC and to obtain the theoretical VS and methane production values. The low deviations obtained (equal to or lower than 10%) between the theoretical and experimental values suggest that the proposed models predict the behaviour of the reactors very accurately.

1. Introduction

The extraction of sunflower oil generates a solid waste with a high organic matter content called sunflower oil cake (SuOC). The high level of production of SuOC in Spain, around 5 million tons a year, is generated during the industrial processing of sunflower seeds into edible oils [1], and could create a serious environmental problem. This by-product can be broken down into three main components: a proteinaceous fraction, a lignocellulosic fraction and a soluble fraction. Moreover, it is edible and has a high nutritional value, especially due to its high protein content. It has been used as animal feed (for animals such as ruminants, poultry and fish) but its high lignocellulosic material content limits this use [1].

Anaerobic digestion can be defined as a biological conversion process without an external electron acceptor, such as oxygen, in the aerobic process. In the anaerobic process organic carbon is converted by subsequent oxidations and reductions to its most oxidized (CO_2) and most reduced (CH_4) states. With the increasing application of the anaerobic digestion process there is an urgent need to establish a method to estimate the biodegradability and methane potential of wastes used in anaerobic digestion [2]. Over the past few years, numerous studies have been carried out in which the biochemical methane potential (BMP) of crop species, wastes and other forms of biomass has been reported [3]. No standard method has been defined for this procedure, however, and even for the study of simpler soluble substrates the conclusions of an international working group are still being awaited. Studies on BMP of complex substrates such as SuOC have not been reported up to now.

Previous works on the effect of inoculum to substrate ratio (ISR) in the BMP assay were limited [4–8]. Reports have also shown a variety of test procedures for the determination of

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anaerobic biodegradability of macro-pollutants and complex substrates, some of these biodegradability tests being introduced after 2002 [2,9–11]. Certain problems related to the equipment and the methodology used (such as permeation phenomena, reproducibility and the medium composition) were addressed. Finally, recommendations were made for improving test equipment and procedures [9–11]. When revising previous works it became clear that two different experimental set-ups are commonly used to establish the biodegradability and methane potential of particulate substrates, i.e. batch [2,12] or continuous [2,13]. In the batch test, the selected substrate (waste) is incubated in closed vials or flasks at a specific temperature with a certain amount of methanogenic inoculum. After incubation the degree of degradation of the substrate is evaluated at pre-set time intervals to determine the rate and ultimate biodegradation or hydrolysis. Controls with only inoculum added are included in order to account for the biogas produced from organic matter contained in the inoculum [12]. The continuous set-up uses completely stirred tank reactors (CSTR) operating at a specific temperature and at different hydraulic retention times (HRT) [13]. Once steady-state has been established, the effluent is analysed. However, the continuous set-up is much more laborious than the batch set-up [2,12]. Batch experiments can be performed in a single-flask batch reactor or a multiple-flask batch reactor [14–16]. The latter is actually a system of several small batch reactors of equal contents that allows more homogenisation than one large batch reactor. This type of batch reactor is mainly used for assessment of biodegradability and hydrolysis rates of low homogeneity wastes such as lipid-containing wastes [2].

There are contradictory results reported in the literature about the influence of ISR on methane yield coefficient [17]. In that sense, previous studies have shown that increasing ISR may affect negatively the ultimate practical methane yield [17]. However, other investigations have shown no significant influence [18]. Organic or inorganic toxicants contained in the waste might inhibit the process, so dilution of the waste might also influence significantly the practical methane potential [17]. There are still no existing reported studies about the anaerobic biodegradability of SuOC. In addition, the BMP of this substrate and the influence of ISR on the methane yield have not been reported in the literature.

Therefore, the aim of this work was to study the anaerobic biodegradability of SuOC and simultaneously to assess the influence of the ISR on the batch anaerobic digestion of this waste, as one of the factors most likely to affect the result of a BMP test carried out in batch mode, and to monitor the progress of the anaerobic conversion by standardized procedures. A kinetic evaluation for substrate degradation and methane generation within the anaerobic processes involved was also carried out.

2. Materials and methods

2.1. Characteristics and features of the substrate

The SuOC sample used in this study was taken from a sunflower oil factory located near Seville (Spain). Prior to using the substrate, it was sieved to give a fraction with a particle size lower than 2 mm.

The characteristics of SuOC are largely determined by the oil extraction process from which it is derived. Variations occur due to differences in seeds, the number of hulls removed in processing and the processing method itself (mechanical or solvent extraction) [18]. The main characteristics and composition of the SuOC used in the experiments were (average values of three determinations with standard deviations): dry matter, $92 \pm 1\%$; moisture, $8.0 \pm 0.5\%$; volatile solids, $93.4 \pm 1.9\%$; ash, $6.6 \pm 0.1\%$; chemical oxygen demand (COD), 1.08 ± 0.04 g O_2 g^{-1} TS dry basis; neutral detergent fibre, $48.4 \pm 2.4\%$; acid detergent fibre, $35.0 \pm 1.8\%$; lignin, $13.0 \pm 0.6\%$; total protein, $31.4 \pm 1.6\%$; fat, $1.7 \pm 0.1\%$; soluble sugars, $2.0 \pm 0.1\%$; polyphenols, $0.70 \pm 0.03\%$; carbon, $43.6 \pm 0.3\%$; hydrogen, $6.2 \pm 0.1\%$; nitrogen, $4.3 \pm 0.6\%$; sulfur, $0.30 \pm 0.05\%$; and oxygen, $45.6 \pm 0.5\%$.

From the elemental composition of C, H, O, N and S (in percentages) of the waste, the following empirical formula for the SuOC was obtained: $C_{363}H_{620}O_{285}N_{31}S$.

2.2. Inoculum

Granular sludge taken from an industrial anaerobic reactor, which was treating brewery wastewater, was used as inoculum. The characteristics and features of the anaerobic sludge used were: pH, 7.6 ± 0.1; total solids (TS), 60 ± 3 g/L; volatile solids (VS), 45 ± 2 g/L; volatile suspended solids (VSS), 44 ± 1 g/L; total alkalinity (TA), 900 ± 45 mg $CaCO_3$/L; and total volatile fatty acids (TVFA), 100 ± 5 mg COD/L. The specific methanogenic activity (SMA) of the inoculum was determined using a mixture of acetic, propionic and butyric acids in the proportion 73:23:4 to give a loading of 2–3 g COD/L [19]. The SMA value obtained was 0.99 g COD/(g VSS d). This value clearly demonstrated the high methanogenic activity of the fresh sludge selected as inoculum.

2.3. Experimental set-up

The experimental study was carried out in a multi-batch reactor system, which consisted of 7 Erlenmeyer flasks, with an effective volume of 250 mL. They were continuously stirred with magnetic bars at 300 rpm and placed in a thermostatic water bath at mesophilic temperature (35 ± 1 °C). For each ISR assayed the operational sequence of the system was: 7 fed reactors and 7 reactor controls. The fed reactors were initially charged with the corresponding amount of substrate and one of them was sacrificed and removed every day to study the evolution of the chemical parameters at various times in the anaerobic digestion process. The methane production due to biomass decay and the possible presence of residual substrate in the inoculum was subtracted by performing blank controls. The reactors were run for only 7 days because no significant methane production was observed after this time.

2.4. Experimental procedure

Table 1 describes the experimental protocol used in the batch anaerobic digestion assays. The six different ISRs tested were: 3.0, 2.0, 1.5, 1.0, 0.8 and 0.5, which were achieved by keeping a constant inoculum concentration (15 g VS/L) and varying the substrate concentration which ranged from 5 to 30 g VS/L. A 20% (v/v) basal medium (50 mL) with macro and micronutrients was used. The composition of this solution is given in detail elsewhere [18]. A 10% (v/v) of a solution of 50 g $NaHCO_3$/L (25 mL) was also added to give a TA of 3.4 g $CaCO_3$/L at the beginning of the process (zero time). Finally, the reactors were filled up to 250 mL with distilled water

Table 1
Experimental conditions used for the different batch anaerobic digestion tests carried out.

Inoculum concentration (g VS/L)	Substrate concentration (g VS/L)	ISR
15	5.0	3.0
15	7.5	2.0
15	10.0	1.5
15	15.0	1.0
15	18.8	0.8
15	30.0	0.5

and the head space flushed with nitrogen. The reactors ran until no further methane production could be detected. The duration of the experiments was 7 days in all cases. This short period of time was long enough to achieve the maximum methane production, and can basically be explained by the high methanogenic activity of the sludge and by the short interval between the taking of the inoculum and the start-up of the experiments (less than 72 h).

3. Analytical methods

3.1. Substrate

Dry matter, moisture, VS and ash were determined according to the standard methods 2540B and 2540E [20].

Total chemical oxygen demand (CODt) was determined using the reported proposed method by Raposo et al. [21]: for this analysis, 100 mg of sample, 20 mL $K_2Cr_2O_7$ 1.2N, 30 mL H_2SO_4–Ag_2SO_4 and the final titrated solution with ferrosous ammonium sulphate 0.5N were used.

Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined according to the procedure of Goering and Van Soest [22].

Total protein was determined by multiplying the total Kjeldahl nitrogen (TKN) value by 6.25 [23]. To determine the TKN, 1000 mg of sample was acidified with 10 mL concentrate H_2SO_4. In addition, 5 g catalyst (9% $CuSO_4{\cdot}5H_2O$–91% K_2SO_4) was added, and finally the sample was digested in a termoblock for 30 min at 150 °C and 75 min at 375 °C. After cooling down, the sample was diluted with 40 mL distilled water, neutralized with NaOH 12.5N and distilled in 40 mL of solution indicator mix. The solution was titrated with H_2SO_4 1N.

The fat content was extracted with hexane, using a soxhlet system [24].

Soluble sugars and polyphenols were determined using the same ethanolyc extract obtained by soxhlet extraction of 2 g of sample with 150 mL of ethanol 95% (v/v). The extract was filtered with Whatman No.1 and transferred to a volumetric flask of 200 mL. Soluble sugars were determined according to the Dubois et al. [25] procedure and polyphenols by the Moores et al. [26] method.

Elemental composition of the SuOC was determined using a Leco CHNS-932 elemental analyzer, following the manufacturer's standard procedures.

3.2. Inoculum and digestate

The pH was measured using a pH-meter model Crison 20 Basic. TS and VS were determined gravimetrically using the standard methods 2540B and 2540E, respectively [20].

The supernatant obtained after centrifuging the samples for 6 min at 10,000 rpm was filtered through a glass microfibre filter and was used to characterize the following parameters: soluble chemical oxygen demand (CODs), TA and TVFA. CODs were measured using the closed digestion and titrimetric standard method 5220 C [20]. TA was measured by pH titration to 4.3 according to Jenkins et al. [27]. TVFA were analysed according to the standard method 5560 C [20].

4. Results and discussion

4.1. Process stability

As can be seen in Fig. 1, the pH ranged from 7.6 to 6.8, the lowest value corresponding to the ISR of 0.5 at 1 day of digestion time. These pH values were compatible with the normal growth

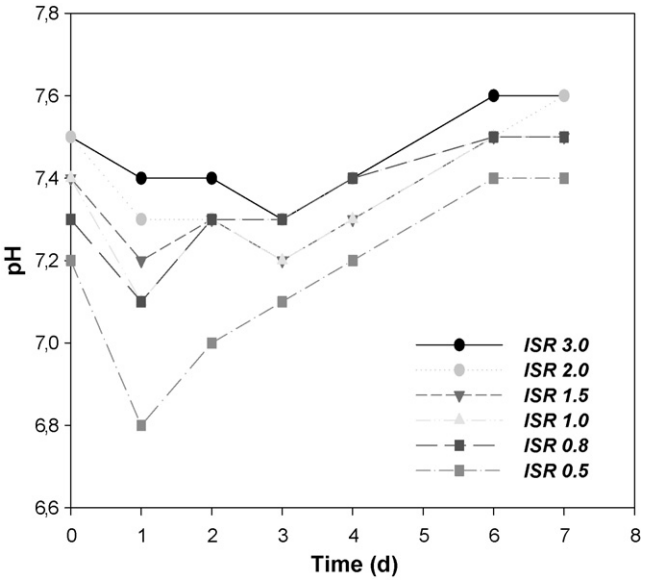

Fig. 1. Variation of the pH of digestates with digestion time for all ISRs assayed.

of anaerobic microorganisms. This means that this parameter was always stable during the anaerobic digestion process. Furthermore, no imbalance was observed in the pH, even when the ISR used was at 0.5. With ISR at 0.5, the highest concentration of TVFA was produced (5500 mg acetic acid/L) which brought methane production to an almost complete halt. Therefore, it can be concluded that this parameter is not a good tool for evaluating the stability of the process, as is pointed out in many other studies [18,28,29]. In addition, the presence of low pH is the result of a well-developed imbalance and as such is not useful as an early warning indicator, as was previously reported in the literature [29].

During the anaerobic acid-phase stage of complex organic substrates, mainly constituted by carbohydrates, proteins and lipids, these are converted basically to volatile fatty acids (VFA) and to a lesser extent to other low molecular weight compounds [30]. CODs is a parameter that represents the extent of solubilisation. In the present study, the initial and final values of CODs were always proportional to the load added [31]. The values of net CODs (subtracting final and initial CODs concentrations) obtained were: 780, 900, 1,900, 1,980, 3,400 and 6,100 mg O_2/L for ISR of 3.0, 2.0, 1.5, 1.0, 0.8 and 0.5, respectively.

The final values of TVFA were proportional to the amount of SuOC added, and no accumulation at the end of the digestion time happened at ISRs of 3.0, 2.0, 1.5 and 1.0. However, for ISRs lower than 1.0, an imbalance of the process was observed, increasing the TVFA concentration up to values of 2050 and 5500 mg acetic acid/L for ISRs of 0.8 and 0.5, respectively [31]. An increase was also observed in the CODs of digestates at the end of digestion time (7 days) achieving final values of 3380, 4000, 5600, 6180, 7900 and 12,100 mg/L for ISRs of 3.0, 2.0, 1.5, 1.0, 0.8 and 0.5, respectively [31]. The trend in the increase in the CODs with digestion time observed (taking into account that the CODs in the blank reactor was 1000 mg O_2/L) was mainly due to the accumulation of VFA, which reflects a kinetic uncoupling between acid formers and consumers and is typical of a stress situation [29]. This means that the hydrolytic-acidogenic stage was carried out satisfactorily and the imbalance of the process was due to the stress of methanogenic microorganisms.

The TVFA/TA ratio can be used as a measure of process stability [32,33]: when this ratio is lower than 0.3–0.4 (equiv. acetic acid/equiv. $CaCO_3$) the process is considered to be operating

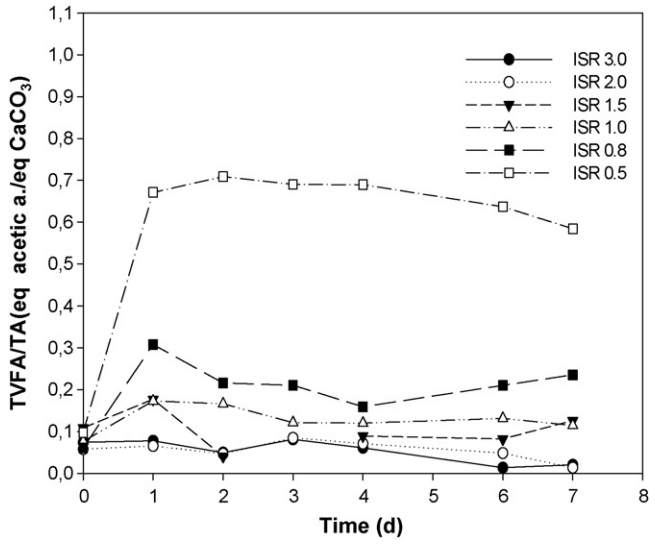

Fig. 2. Variation of the total volatile fatty acids (TVFA)/total alkalinity (TA) ratio with digestion time for all ISRs studied.

favourably without the risk of acidification. As can be seen in Fig. 2, the ratio values were lower than the suggested limit value for ISRs equal to or higher than 0.8, which demonstrated the high stability of all assays carried out except for the experiment corresponding to an ISR of 0.5. For this ISR, the TVFA/TA ratio was around 0.7 during all digestion times, which clearly showed the destabilisation of this digester. This increase in the TVFA/TA ratio was concomitant with the decrease in the pH, which achieved a value of 6.8 at 2 days of digestion time (Fig. 1).

4.2. Kinetic evaluation

Assuming that the volatile solids content derived from the substrate is made up of two fractions: one biodegradable and another non-biodegradable or refractory to be biodegraded, the following equation is obtained:

$$VS_T = VS_B + VS_{NB} \quad (1)$$

where VS_T = total volatile solids; VS_B = biodegradable volatile solids; VS_{NB} = non-biodegradable volatile solids.

In order to describe the evolution of the volatile solids content with time, the following differential equation is proposed:

$$\frac{-d[VS_B]}{dt} = K \cdot X \cdot [VS_B] \quad (2)$$

where X is the biomass concentration, K a kinetic constant and t the digestion or operation time.

Eq. (2) can be integrated assuming that X remains virtually constant, given that the low level of growth of the biomass during the operation time is compensated by the endogenous metabolism [29,30,34]. Assuming this hypothesis, separating variables and then integrating, the following equation is obtained:

$$Ln\left\{\frac{[VS_B]_0}{[VS_B]_t}\right\} = K \cdot X \cdot t \quad (3)$$

where the subscripts *0* and *t* denote the biodegradable volatile solids at time zero and time *t*, respectively.

By substituting Eq. (3) into Eq. (1) and operating, the following equation is obtained:

$$[VS_T]_t = VS_{NB} + \{[VS_T]_0 - VS_{NB}\} \cdot e^{-KXt} \quad (4)$$

Table 2
Values of the fraction of non-biodegradable volatile solids and apparent kinetic constants, K_1, for the VS degradation or hydrolysis process with their corresponding standard deviations (σ) for all ISRs studied. These parameters were obtained by fitting the experimental data to Eq. (5).[a]

ISR	$[VS_{NB}] \pm \sigma$ (mg/L)	$K_1 \pm \sigma$ (d^{-1})
3.0	1901 ± 254	0.54 ± 0.09
2.0	3510 ± 122	0.63 ± 0.09
1.5	5377 ± 88	0.43 ± 0.03
1.0	7866 ± 483	0.35 ± 0.06
0.8	10548 ± 243	0.39 ± 0.04
0.5	17334 ± 429	0.32 ± 0.03

[a] Probability level of 95% ($p < 0.05$).

Eq. (4) can be converted into the following simpler equation:

$$[VS_T]_t = VS_{NB} + \{[VS_T]_0 - VS_{NB}\} \cdot e^{-K_1 t} \quad (5)$$

because all digesters contain the same biological sludge or biomass concentration (X = 15 g VS/L), the apparent kinetic constant K_1 being equal to $K_1 = K \cdot X$.

The adjustment by non-linear regression of the pairs of experimental data (t, $[VS_T]_t$) using the Sigmaplot software (version 9.0) allows the calculation of the fraction of non-biodegradable volatile solids, VS_{NB}, and the apparent kinetic constant K_1.

Assuming that a proportional relationship between the degraded volatile solids and the methane generated occurs, the following equation between the two parameters can be formulated:

$$[VS_B]_0 - [VS_B]_t = Yp \cdot G \quad (6)$$

where Y_p is the methane yield coefficient and G the volume of methane gas accumulated at time t.

When $t \to \infty$; $[VS_B]_t \to 0$ and $G \to G_m$ and, therefore, Eq. (6) can be transformed into:

$$[VS_B]_0 = Yp \cdot G_m \quad (7)$$

Finally, by substituting Eqs. (6) and (7) into Eq. (3) the following mathematical expression is obtained:

$$G = G_m(1 - e^{K_2 \cdot t}) \quad (8)$$

where G_m is the maximum methane volume obtained at an infinite digestion time, and K_2 is the apparent kinetic constant for methane production.

The kinetic constant K_2 is different from K_1 because the hydrolysis and methanogenesis are carried out by different microorganism groups.

Fig. 3a–c shows the variations of the VS_T concentration and methane generated (G) with time for the experiments corresponding to ISRs of 3.0, 2.0 (Fig. 3a), 1.5, 1.0 (Fig. 3b) and 0.8, 0.5 (Fig. 3c), respectively. These Figures simultaneously plot both the experimental values (points) and the theoretical or simulated curves obtained by Eqs. (5) and (8), respectively. As can be seen, the VS_T decreases gradually with time until it reaches an asymptotic value, which is not equal to zero, as a consequence of the occurrence of compounds that are not anaerobically biodegradable. This coincides with the stop in methane production. Therefore, in accordance with these results, the proposed Eq. (5) can be applied to the experimental results. By non-linear regression using the SigmaPlot software (version 9.0) the fraction of non-biodegradable volatile solids VS_{NB} and the apparent kinetic constant K_1 were calculated. Table 2 summarizes the values of the apparent kinetic constants for volatile solids degradation, K_1, and the fractions of non-biodegradable volatile solids VS_{NB} with their corresponding standard deviations for all ISRs studied. The low standard deviation values for these two parameters show an adequate fit of the experimental data to the proposed model.

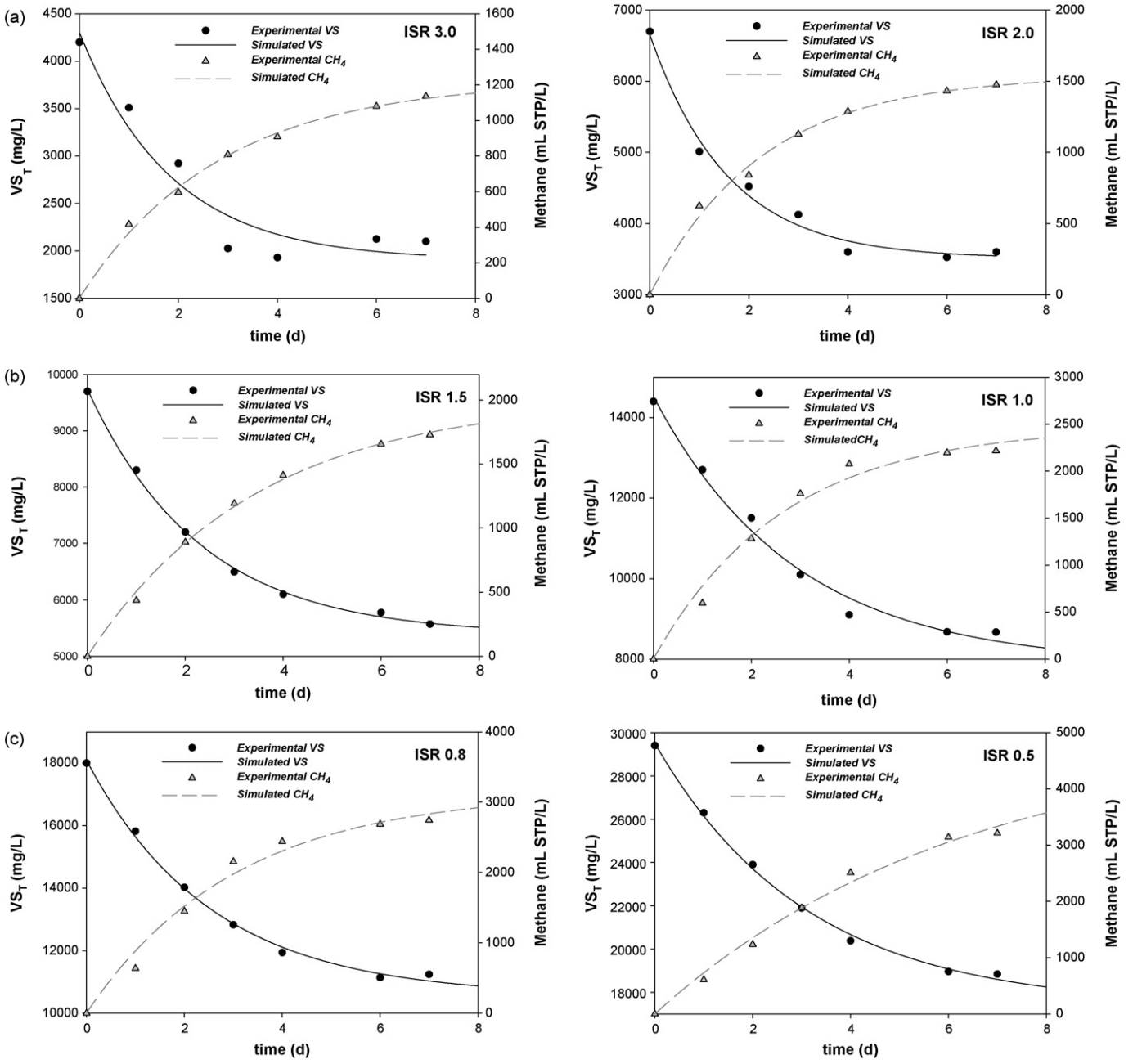

Fig. 3. Variation of the VS_T concentration and methane production with digestion time for the ISRs of 3.0 and 2.0 (a), ISR of 1.5 and 1.0 b) and ISR of 0.8 and 0.5 (c). The solid curves plotted correspond to the theoretical or simulated VS_T and methane production obtained by Eqs. (5) and (8), respectively.

In addition, according to Eq. (8), methane production conforms to a first order kinetic model [35,36]. As can be seen in Fig. 3a–c, G was zero at $t = 0$ and the rate of gas production became zero at $t =$ infinite. As expected, the amount of gas produced rose by increasing the load or by decreasing the ISR. The slopes of the curves decreased with increasing digestion time. This drop can be attributed to the gradual decrease in the concentration of biodegradable substrate. Eq. (8) complies well with the experimental data. Thus, it seems reasonable to apply the proposed kinetic model to all ISRs assayed. The values of K_2 and G_m for each ISR studied were calculated numerically from the experimental data by non-linear regression using the Sigmaplot software (version 9.0). Table 3 shows the K_2 and G_m values obtained and their standard deviations. As can be seen, the standard deviations of G_m and K_2 were less than 10% and 5%, respectively, for all of the ISRs studied, suggesting that the proposed model fits to the experimental data adequately.

Figs. 4 and 5 show the variation of the apparent kinetic constants K_1 and K_2, respectively, with the ISR. As can be seen, both kinetic constants dropped markedly with decreasing ISR or with increasing substrate concentration in the reactor, showing the occurrence of an inhibition process. Specifically, the K_1 value decreased 1.7 times when the ISR decreased from 3.0 to 0.5, while K_2 decreased 2.2 times within this same ISR range. For an ISR of 0.5, the value of the kinetic constant K_2 ($0.16 \pm 0.03\ d^{-1}$) was significantly lower than that obtained for K_1 ($0.32 \pm 0.03\ d^{-1}$) as a consequence of the high accumulation of TVFA, which brought about a considerable decrease in the pH value (Figs. 1 and 2). A similar behaviour

Table 3
Values of the maximum methane generation, G_m, and apparent kinetic constants, K_2, for methane formation with their corresponding standard deviations (σ) for all ISRs studied. These parameters were obtained by fitting the experimental data to Eq. (8).[a].

ISR	$G_m \pm \sigma$ (mL/L)	$K_2 \pm \sigma$ (d^{-1})
3.0	1225 ± 40	0.36 ± 0.03
2.0	1540 ± 48	0.44 ± 0.04
1.5	2013 ± 73	0.29 ± 0.02
1.0	2475 ± 159	0.38 ± 0.06
0.8	3145 ± 256	0.33 ± 0.06
0.5	4966 ± 702	0.16 ± 0.03

[a] Probability level of 95% ($p < 0.05$).

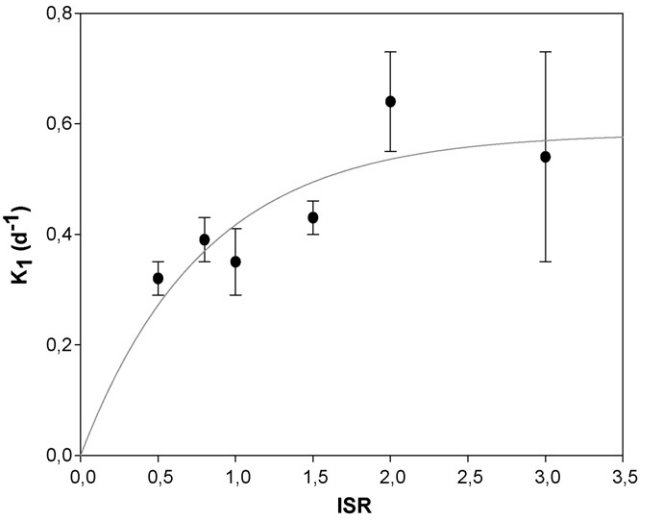

Fig. 4. Variation of the apparent kinetic constant, K_1, for VS_T degradation as a function of the ISR.

has been observed in the anaerobic digestion of wastewater produced in the manufacture of cellulosic pulp from wheat straw [37], untreated molasses [38] and two-phase olive mill effluents [39] in batch reactors.

As can be observed in Table 2, the concentration of non-biodegradable volatile solids is proportional to the initial total volatile solids content present in the reactors. Fig. 6 illustrates the

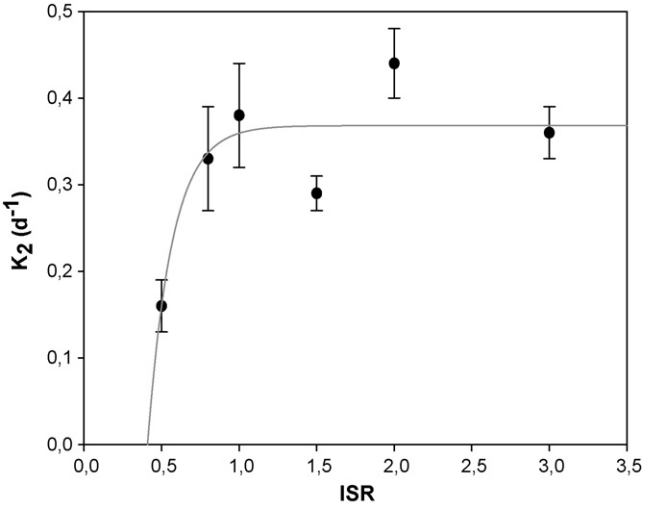

Fig. 5. Variation of the apparent kinetic constant, K_2, for methane production as a function of the ISR.

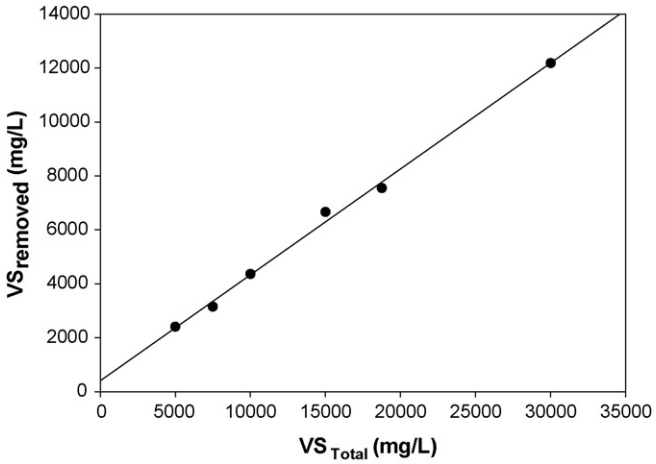

Fig. 6. Amount of substrate removed against substrate added (VS_T) for all the experiments carried out to obtain the percentage of biodegradability of the waste.

variation of the VS removed with the total VS content for the different ISRs tested. The following linear correlation between these two variables was obtained:

$$VS_{removed} = 402 + 0.393\,VS_{Total} (R_2 = 0.996)$$

This confirms that for this substrate, approximately 40% of the total volatile solids are anaerobically biodegradable. This low value can be explained, among other reasons, by the high content of lignine, hemicellulosic and some phenolic compounds present in the SuOC.

The yield coefficients of methane, Y_p (mL CH_4 STP/g $VS_{removed}$) were determined from the experimental data on maximum methane volume produced at the end of digestion time (7 days) and the final and initial VS. Fig. 7 shows the variation of Y_p values with the ISR. By fitting (Y_p, ISR) value pairs corresponding to the different assays carried out, the following equation can be obtained:

$$Y_p = 484[1 - \exp(-1.76(ISR))]$$

This equation demonstrated the marked influence of the ISR on Y_p values and could be used for predicting the methane yield coefficients for different ISRs.

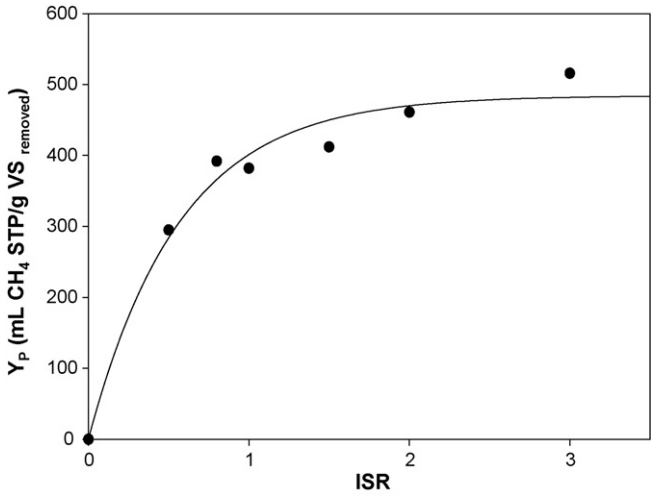

Fig. 7. Variation of the methane yield coefficients, Y_p, with the ISR.

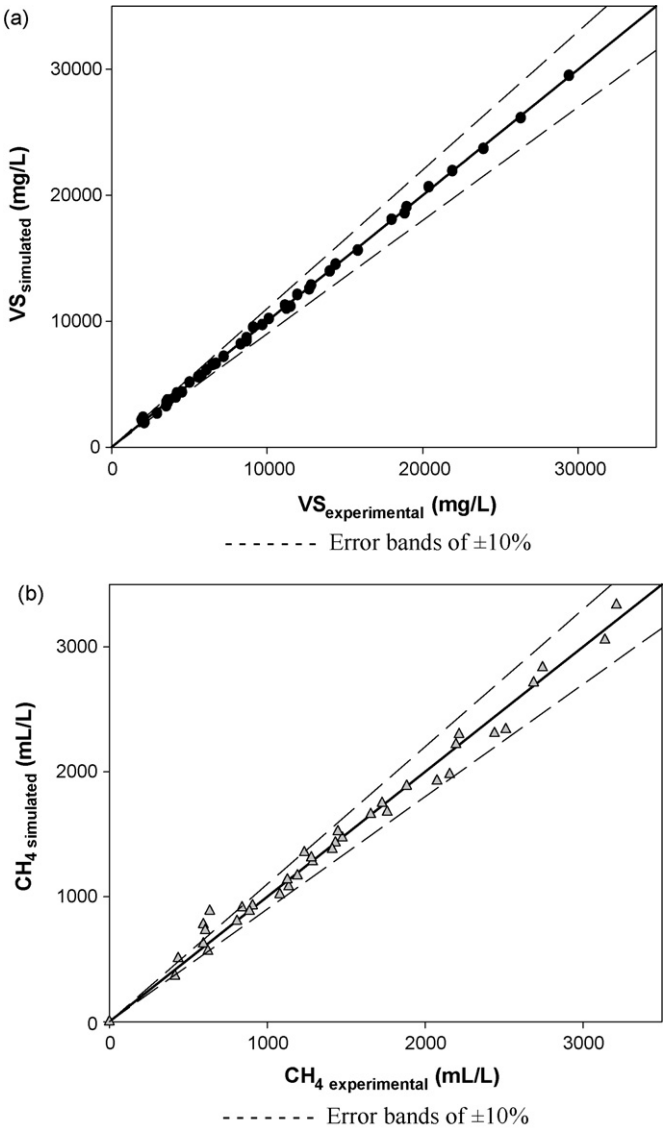

Fig. 8. Comparison between experimental VS_T and the theoretical values predicted by Eq. (5) (a) and comparison between experimental methane production and the theoretical values predicted by Eq. (8) (b).

4.3. Validation of the kinetic models

The proposed kinetic Eqs. (5) and (8) were validated by comparing the theoretical curves with the corresponding experimental data of VS and methane production. Fig. 8a and b shows the comparison of the experimental data of VS (Fig. 8a) and methane production (Fig. 8b) with the corresponding theoretical values obtained by the above equations. These calculations were performed so as to give an error band of ±10% in both cases. As can be seen, the deviations obtained were lower than 10% in practically all cases, suggesting that the proposed models can be used to very accurately predict the behaviour of these reactors. Furthermore, the kinetic parameters obtained represent the activity of the microorganisms effecting the anaerobic digestion of this substrate.

5. Conclusions

The results of this study demonstrate the adequate stability of the batch mesophilic (35 °C) anaerobic digestion process of SuOC at ISRs in the range of 3.0–0.8. pH values in the range of 7.1–7.6 and TVFA/TA ratio values lower than the failure limit range (0.3–0.4) corroborate the appropriate stability of this anaerobic digestion process. Two kinetic models for substrate (VS) degradation and methane production were proposed and evaluated for describing the hydrolysis of the substrate and gas generation. The apparent kinetic constants for solids degradation (K_1) and methane production (K_2) decreased from 0.54 ± 0.09 to $0.32 \pm 0.03\ d^{-1}$ and from 0.36 ± 0.04 to $0.16 \pm 0.03\ d^{-1}$, respectively, when the ISR decreased from 3.0 to 0.5, showing the occurrence of an inhibition phenomenon by substrate concentration. The kinetic equations obtained were used to simulate the anaerobic digestion process of SuOC and to obtain the theoretical VS and methane production values. The low deviations obtained between the theoretical and experimental values (equal to or lower than 10%) suggest that the proposed models predict the behaviour of the reactors very accurately.

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