

Anaerobic Digestion of Wastewater Derived from the Pressing of Orange Peel Generated in Orange Juice Production

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A study of the anaerobic digestion of wastewater from the pressing of orange peel generated in orange juice production was carried out in a laboratory-scale completely stirred tank reactor at mesophilic temperature (37 °C). Prior to anaerobic treatment the raw wastewater was subjected to physicochemical treatment using aluminum sulfate as a flocculant and to pH reduction using a solution of sulfuric acid. The reactor was batch fed at COD loads of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g of COD. The process was very stable for all of the loads studied, with mean pH and alkalinity values of 7.5 and 3220 mg of CaCO₃/L, respectively. The anaerobic digestion of this substrate was found to follow a first-order kinetic model, from which the specific rate constants for methane production, K_G , were determined. The K_G values decreased considerably from 0.0672 to 0.0078 L/(g h) when the COD load increased from 1.5 to 5.0 g of COD, indicating an inhibition phenomenon in the system studied. The proposed model predicted the behavior of the reactor very accurately, showing deviations of <5% between the experimental and theoretical values of methane production. The methane yield coefficient was found to be 295 mL of CH₄ STP/g of COD removed, whereas the mean biodegradability of the substrate (TOC) was 88.2%. A first-order kinetic model for substrate (TOC) consumption allowed determination of the specific rate constants for substrate uptake, K_C , which also decreased with increasing loading, confirming the above-mentioned inhibition process. Finally, the evolution of the individual volatile fatty acid concentrations (acetic, C2; propionic, C3; butyric, C4; isobutyric, iC4; valeric, C5; isovaleric, iC5; and caproic, C6) with digestion time for all loads used was also studied. The main acids generated were acetic and propionic for all loads studied, facilitating the conversion into methane.

KEYWORDS: Anaerobic digestion; wastewater from pressing of orange peel; mesophilic temperature; kinetic constants; biodegradability; methane yield coefficient; volatile fatty acids

INTRODUCTION

Orange cultivation is a major industry and an important part of the economies of the United States (Florida and California), Brazil, Mexico, Pakistan, China, India, Iran, and most Mediterranean countries including Spain and Greece (*1*). According to FAOSTAT, Spanish orange production in 2005 was 2 149 900 tonnes, Spain being the seventh largest producer of oranges in the world (*1*).

Products made from oranges include (*1–5*) orange juice; sweet orange oil, a byproduct of the juice industry produced by pressing the peel or rind (it is used as a flavoring for food and drink and for its fragrance in perfume and aromatherapy;

sweet orange oil consists of about 90% D-limonene, a solvent used in various household chemicals such as furniture polish, and with other citrus oils for grease removal and as a hand-cleansing agent; it is an efficient cleaning agent that is environmentally friendly and much less toxic than petroleum distillates); the orange blossom, the petals of which can also be made into a delicately citrus-scented version of rosewater; orange blossom honey, or actually citrus honey, which is highly prized and tastes much like orange; marmalade, a conserve made usually with Seville oranges (all parts of the orange are used to make marmalade; the pith and pips are separated and typically placed in a muslin bag, where they are boiled in the juice (with sliced peel) to extract their pectin, aiding the settling process).

In the process of orange juice production a large volume of orange peel is generated, which in turn is used as a raw material in the manufacture of some cattle feeds. The first step in this is the pressing of the rind; in the pressing process calcium

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hydroxide is used as binder, and significant amounts of wastewater are intermittently generated. These are very polluting due to the high concentration of organic matter (150 g of COD/L) and high alkalinity.

On the other hand, the ability of orange peel to remove Zn, Ni, Cu, Pb, and Cr from aqueous solution by adsorption was previously studied and reported (3). The adsorption was in the order of Ni(II) > Cu(II) > Pb(II) > Zn(II) > Cr(II). The extent of removal of Ni(II) was found to be dependent on adsorbent dose, initial concentration, pH, and temperature. The adsorption followed first-order kinetics. The process was endothermic, showing monolayer adsorption of Ni(II), with a maximum adsorption of 96% at 50 °C for an initial concentration of 50 mg/L at pH 6. The ability of waste orange peel for the adsorptive removal of methylene blue was also investigated at different initial dye concentrations, agitation time, adsorbent dosage, and pH (4). Adsorption equilibrium data obeyed the Freundlich isotherm model. Adsorption kinetics showed first-order rate expression by Lagergren. A maximum removal of 74% of the dye was observed at pH 10.4. Desorption studies showed that the adsorption was mainly due to ion exchange (4). In the same way, orange peel was recently used to remove textile dyes from aqueous solutions (5). Direct Red 23 (DR23) and Direct Red 80 (DR80) were used as model compounds. The adsorption capacities were 10.72 and 21.05 mg/g at initial pH 2. The effects of initial dye concentrations (50, 75, 100, and 125 mg/L), pH, mixing rate, contact time, and quantity of orange peel have been studied at 25 °C (5). This study demonstrated that the Langmuir equation fit the experimental results better than the Freundlich equation. The results indicated that acidic pH supported the adsorption of both dyes on the adsorbent. Orange peel with concentrations of 8 and 4 g/L has shown adsorption efficiencies of 92 and 91% for DR23 and DR80, respectively. Furthermore, the adsorption kinetics of both dyes was studied, and the rates of sorption were found to conform to pseudo-second-order kinetics with a good correlation ($R^2 = 0.998$) (5).

Because of the high organic content of these wastewaters, anaerobic digestion seems to be the most appropriate process for treatment and purification. Anaerobic treatment of medium- and high-strength wastewaters with a high biodegradable content has a number of advantages (6–9); for example, a high degree of purification can be achieved, nutrient requirements are low, the quantity of excess sludge produced is usually small, and the biogas produced can be used for energy generation or recovery; this can reduce operational costs by a large margin compared with energy-intensive aerobic processes (9).

The aim of the current work was to carry out an evaluation of the performance and stability of anaerobic digestion of the wastewater derived in the pressing of orange peel generated in orange juice production. The study was carried out in batch mode in a laboratory-scale completely stirred tank reactor at mesophilic temperature (37 °C). Because the wastewater to be treated contains high amounts of total and suspended solids, it was first subjected to a physicochemical treatment with aluminum sulfate, with the aim of removing part of the solids content that would hinder and slow its anaerobic treatment. Different kinetic models for methane production and substrate utilization were derived from the experimental data. The proposed models allowed very accurate prediction of the behavior of the system.

MATERIALS AND METHODS

Equipment. The experimental setup used for the anaerobic digestion of wastewater derived in the pressing of orange peel generated in orange juice production consisted of a 1-L Pyrex flask with four connections:

Table 1. Composition and Characteristics of Raw Wastewater Derived from the Pressing of Orange Peel and of Wastewater Treated with Aluminum Sulfate To Remove Part of Its Total and Suspended Solids Contents and Slightly Acidified with Sulfuric Acid after the Addition of N and P

parameter	raw waste water	pretreated wastewater used as substrate
pH	11.21	5.50
alkalinity (mg of CaCO ₃ /L)	8360	1550
volatile acidity, VA (mg of acetic acid/L)	695	600
total chemical oxygen demand, COD (mg/L)	147680	130040
soluble COD, COD _s (mg/L)	140300	128740
total organic carbon, TOC (mg/L)	52970	47395
total suspended solids, TSS (mg/L)	20780	17030
mineral suspended solids, MSS (mg/L)	3200	1230
volatile suspended solids, VSS (mg/L)	17580	15800
total solids, TS (mg/L)	151900	138025
mineral solids, MS (mg/L)	14160	14225
volatile solids, VS (mg/L)	137740	123800
PO ₄ ³⁻ (mg/L)		2.61
N-NO ₃ ⁻ (mg/L)		7.23
N-NH ₄ ⁺ (mg/L)		56.7

Table 2. Composition of Nutrient and Trace Element Solutions

nutrient solution	
NH ₄ Cl	1.4 g/L
KH ₂ PO ₄	1.25 g/L
MgSO ₄ ·7H ₂ O	0.5 g/L
CaCl ₂	0.04 g/L
NaNO ₃	2.0 g/L
yeast extract	0.5 g/L
trace element solution	
FeCl ₂ ·4H ₂ O	2000 mg/L
CoCl ₂ ·6H ₂ O	2000 mg/L
MnCl ₂	318 mg/L
CuCl ₂	24 mg/L
ZnCl ₂	50 mg/L
H ₃ BO ₃	50 mg/L
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	90 mg/L
Na ₂ SeO ₃	68 mg/L
NiCl ₂ ·6H ₂ O	50 mg/L
EDTA	1000 mg/L
HCl 36%	1 mL/L
resazurine	500 mg/L

one in the top and the other three in the side. These were used for loading feedstock, venting the biogas, passing inert gas (nitrogen) to maintain the anaerobic conditions, and removing effluent. The flask contents were stirred magnetically, and temperature was maintained by means of a thermostatic jacket through which water at 37 °C was circulated.

The volume of methane produced in the process was measured by using a 1-L Boyle–Mariotte reservoir connected to the reactor. To remove the CO₂ produced, a tightly closed bubbler containing a NaOH solution (6 N) was connected between the two elements. The methane displaced a measurable quantity of water from the reservoir equivalent to the volume of methane produced.

Inoculum. The reactor was inoculated with methanogenically active biomass obtained from a full-scale anaerobic reactor treating brewery wastewater in the Heineken SA factory (Jaen, Spain). The inoculum was selected on the basis of its high methanogenic activity, ranging between 0.87 and 0.99 g of COD/(g of VSS day) (10).

Wastewater. Table 1 shows the characteristics of the raw wastewater derived from the pressing of orange peel generated in orange juice production. Given the high total and suspended solids content of the raw wastewater, prior to anaerobic treatment it was subjected to a physicochemical treatment using aluminum sulfate as flocculant (at a concentration of 100 mg/L) and to pH reduction using a solution of sulfuric acid. Finally, nitrogen (as NH₄Cl) and phosphorus (as KH₂-

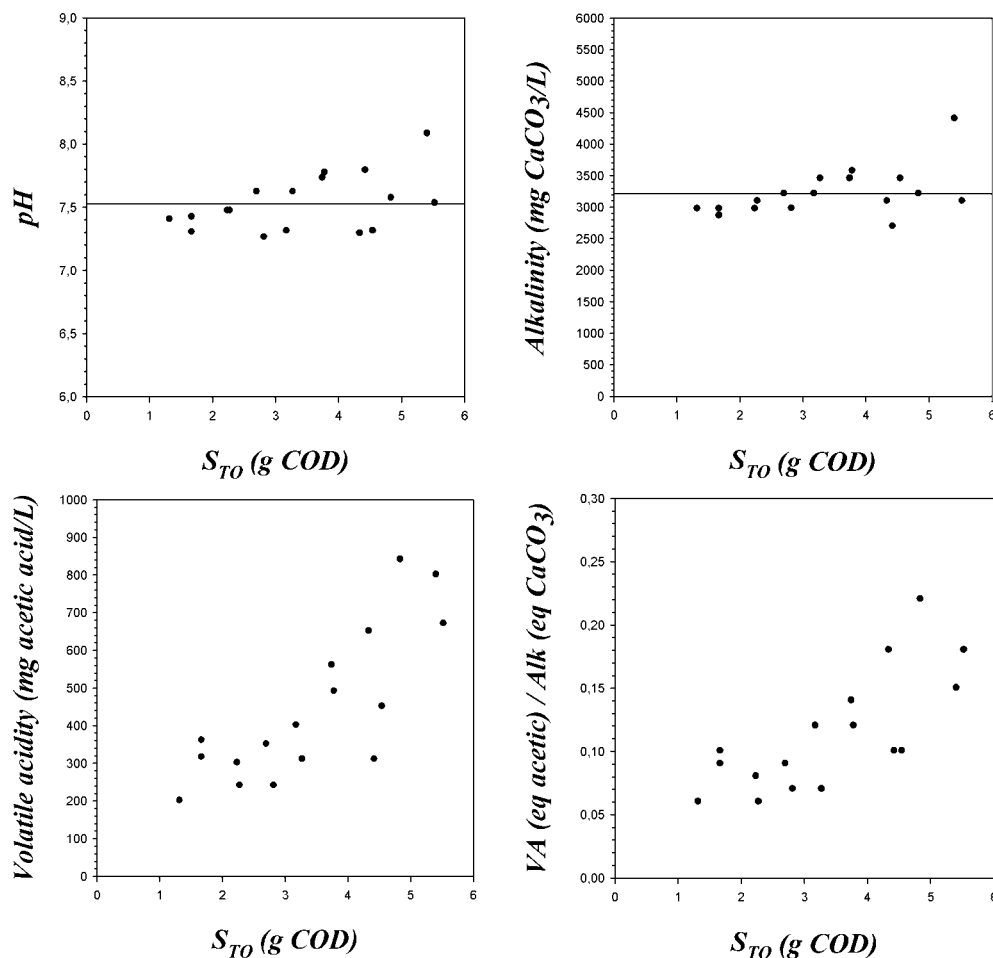


Figure 1. Variation in pH, alkalinity, volatile acidity, and volatile acidity/alkalinity ratio in the effluents of the reactor as a function of the load added, S_{TO} (grams of COD).

PO_4) were added to the final wastewater to be anaerobically digested, with the aim of providing the nutrients necessary for the appropriate metabolism of the microorganisms involved in the process. **Table 1** shows the characteristics of this pretreated wastewater used as substrate in the anaerobic digestion experiments.

Experimental Procedure. The anaerobic reactor was initially loaded with the above-described inoculum (7 g of VSS), 200 mL/L of a nutrient element solution, and 5 mL/L of a trace element solution, the latter two being important in activating bacterial growth and metabolism at the start of the process (9, 11). The compositions of the nutrient and trace element solutions are given in **Table 2**.

With the aim of activating the biomass, prior to the start of the experiments, the reactor was first fed with a synthetic solution composed of glucose, sodium acetate, and lactic acid (GAL solution) at concentrations of 50 g/L, 25 g/L, and 20.8 mL/L, respectively. During this initial period, the organic load added to the reactor was gradually increased from 0.25 to 1.50 g of COD over a 10-day period. Finally, after this previous stage, and with the objective of acclimatizing the biomass to the substrate, before the start of the experiments, the reactor was fed with four loads of 1 g of COD in which the percentage of wastewater to synthetic solution was increased from 25 to 100%. During this acclimatization period, the volume of methane was measured as a function of time. The duration of each assay was 48 h, equal to the time required for the complete biomethanization of each load.

Once this preliminary acclimatization step was completed, a series of batch experiments were carried out using the pretreated wastewater as substrate. During the experiments, the organic load added to the reactor was gradually increased from 1.5 to 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g of COD. In all cases, the volume of methane was measured as a function of time, and the initial and final COD, TOC, pH, volatile acidity, alkalinity, TSS, VSS, TS, and VS values were determined. The duration of each experiment was the time interval required to achieve

maximum cumulative gas production and COD removal from each load, which was found to be in the range from 48 to 72 h. All experiments were carried out in duplicate and the results expressed as means.

Chemical Analyses. The following parameters were determined in the effluent at each loading: pH, COD, COD_s , TOC, TS, MS, VS, TSS, MSS, VSS, volatile acidity (VA), and alkalinity. All analyses were carried out in accordance with standard methods (12).

For TOC determination a Rosemount Analytical Dohrmann DC-190 carbon analyzer was used. The TOC analyzer was calibrated with a standard solution of potassium phthalate prior to the TOC analyses.

Separate volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids) were determined using a Hewlett-Packard HP-560 gas chromatograph equipped with a 15 m \times 0.53 mm (i.d.) Nukol-silica semicapillary column and a flame ionization detector. The oven temperature was gradually increased from 100 to 150 °C at a rate of 4 °C/min. Helium (28.6 kPa), nitrogen (28.6 kPa), hydrogen (14.3 kPa), and air (28.6 kPa) were used as carrier gas at a flow rate of 50 mL/min.

RESULTS AND DISCUSSION

Control Parameters and Reactor Stability. As can be seen in **Table 1**, TS and TSS removals of 9.1 and 18.0%, respectively, were observed when the wastewater was previously treated with aluminum sulfate and sulfuric acid. Thus, although the percentage of solids removal was not high, this pretreatment produced a more homogeneous wastewater for anaerobic digestion.

Figure 1 shows the evolution of the pH, alkalinity, volatile acidity, and volatile acidity/alkalinity ratio in the effluents of the reactor with the load added, S_{TO} (grams of COD). As can

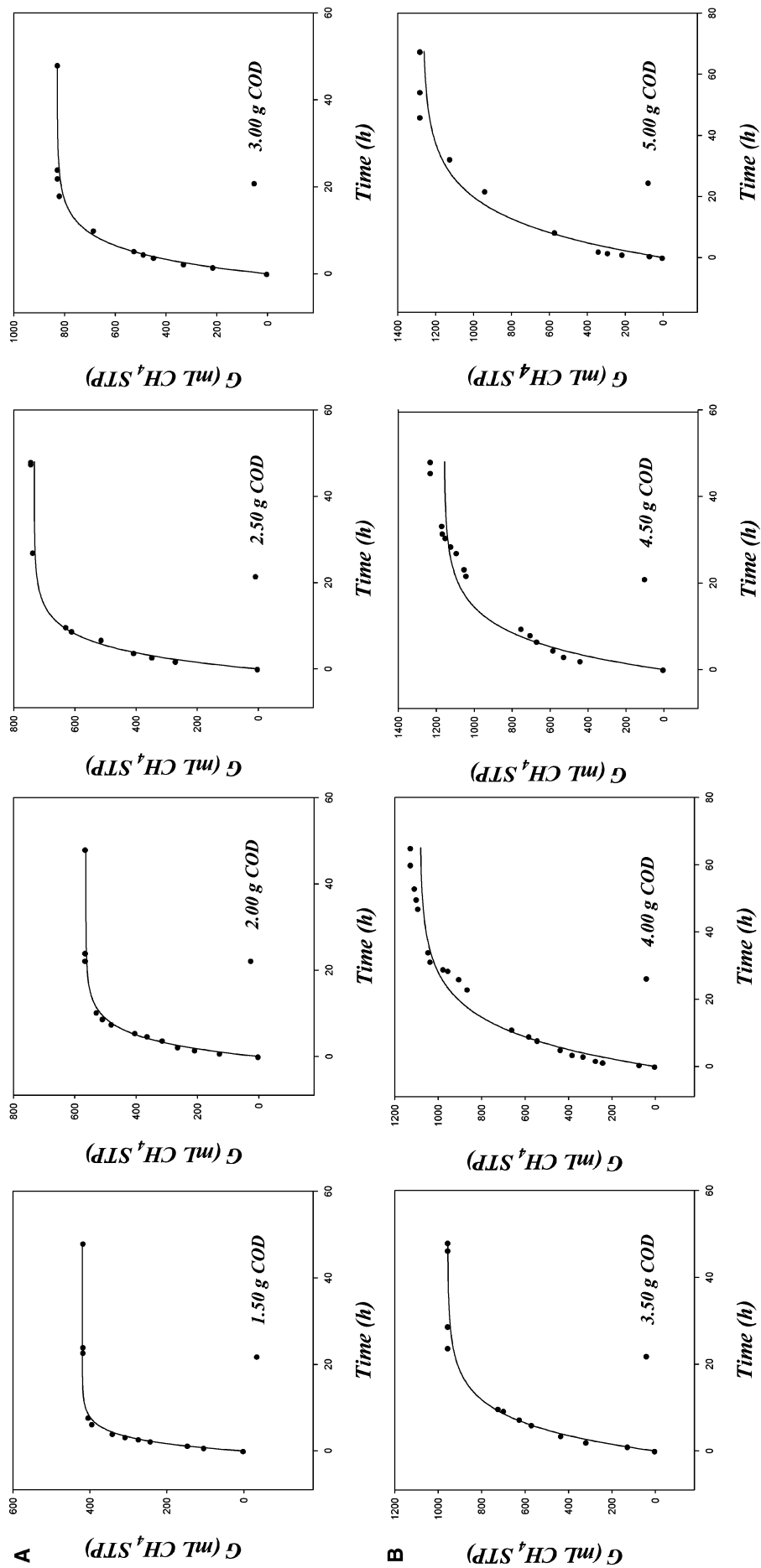


Figure 2. Variation in the volume of methane accumulated (G) as a function of time for loads of (A) 1.5, 2.0, 2.5, and 3.0 g of COD and (B) 3.5, 4.0, 4.5, and 5.0 g of COD.

Table 3. G_m and K_G' Values with Standard Deviations Obtained for All Loads Studied

	load							
	1.5 g of COD	2.0 g of COD	2.5 g of COD	3.0 g of COD	3.5 g of COD	4.0 g of COD	4.5 g of COD	5.0 g of COD
G_m (mL of CH_4 at STP)	420 ± 4	564 ± 11	733 ± 14	828 ± 9	956 ± 15	1084 ± 24	1157 ± 30	1443 ± 99
K_G' (h^{-1})	0.386 ± 0.011	0.246 ± 0.014	0.210 ± 0.014	0.198 ± 0.006	0.153 ± 0.007	0.091 ± 0.007	0.138 ± 0.013	0.040 ± 0.006
R^2	0.9973	0.9889	0.9912	0.9970	0.9938	0.9768	0.9581	0.9705

Table 4. Variation of VSS Content of the Reactor with Loading

load (g of COD)	VSS (mg/L)	load (g of COD)	VSS (mg/L)
1.5	5775	3.5	4808
2.0	5700	4.0	4833
2.5	5642	4.5	4959
3.0	5146	5.0	5534

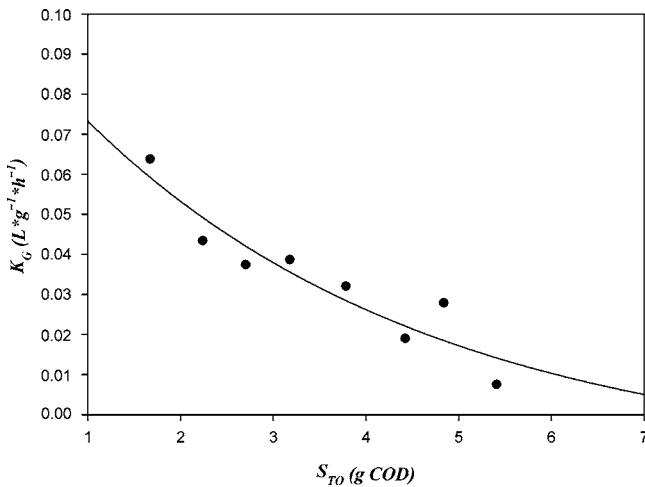


Figure 3. Variation in the specific rate constant, K_G , with the initial COD (S_{T0}).

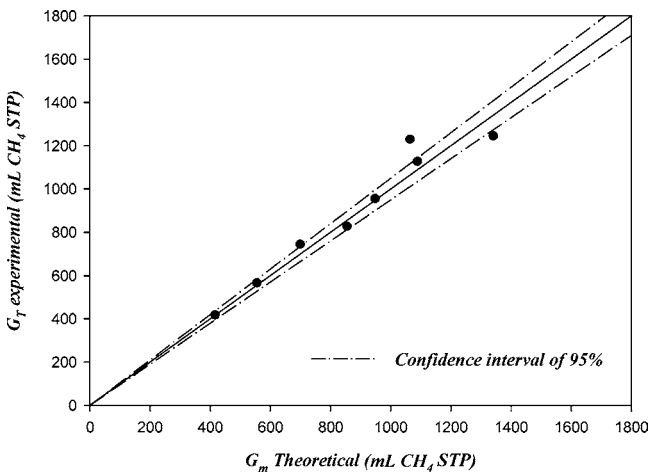


Figure 4. Comparison between experimental maximum methane production (G_T) values and theoretical values (G_m) predicted by eq 1.

be seen, the pH in the reactor remained approximately constant at all of the applied loadings, with a mean value of 7.5, which is within the optimal range for methanogenic bacteria (9, 11), and with extreme values of 7.3 and 7.8. The buffering capacity of the experimental system was maintained at favorable levels with excessive total alkalinity present at all loadings, at a mean value of 3220 mg of $CaCO_3/L$. This buffering protects against possible acidification of the reactor, giving a pH of the same order as the optimal for methanogenic microorganisms (11). The pH and buffering capacity protects against acidification of

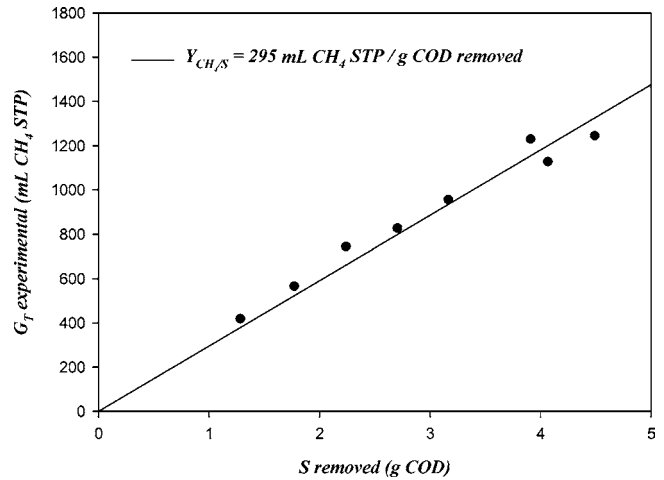


Figure 5. Variation in experimental maximum methane volume produced (G_T) with the COD consumed to obtain the methane yield coefficient of the process.

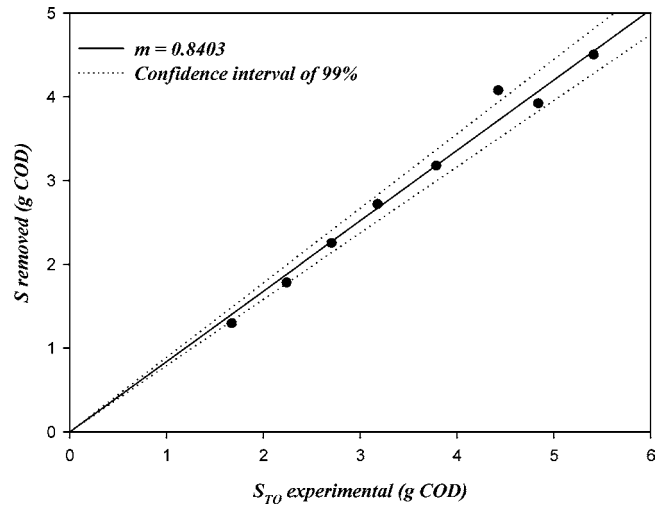


Figure 6. Amount of substrate removed against substrate added for all of the experiments carried out to obtain the percentage biodegradability of the waste.

the reactor, which could be caused by a sudden overloading reactor, an abrupt change in operating temperature, or the presence of toxic compounds or inhibitors in the substrate (13, 14).

Volatile acidity increased from 200 to 900 mg of acetic acid/L when the load added increased from 1.5 to 5.0 g of COD, with a mean value of 400 mg of acetic acid/L. The volatile acidity/alkalinity ratio can be used as a measure of process stability (15): when this ratio is <0.3–0.4, the process is considered to be operating favorably without the risk of acidification. **Figure 1** shows the variation of this ratio as a function of the reactor loading (grams of COD). As can be seen, although the ratio increased with increasing load, it was always lower than the suggested limit value, indicating the stability of the reactor. Moreover, the volatile acidity/alkalinity ratio during the opera-

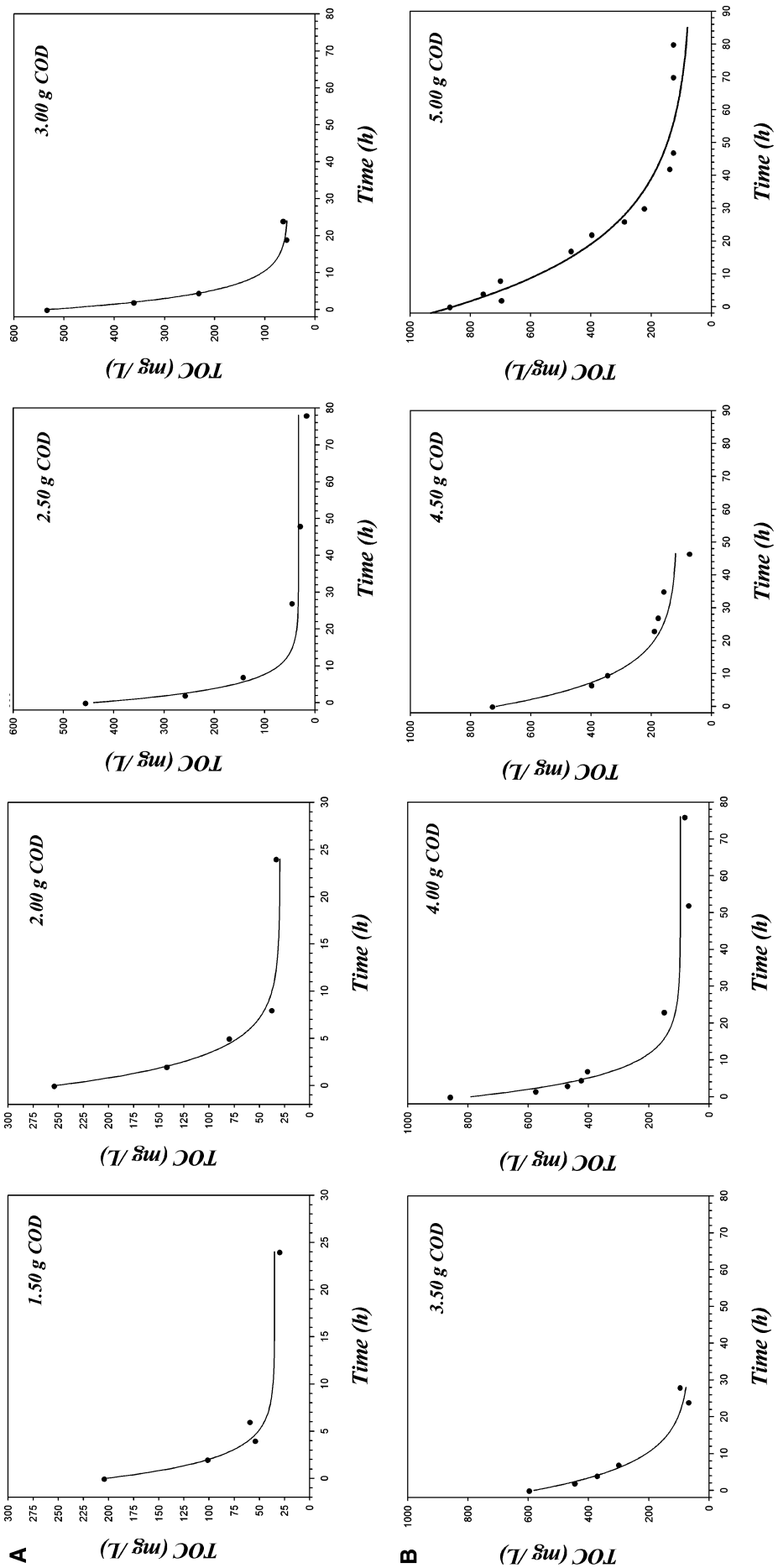


Figure 7. Variation in total organic carbon (TOC in milligrams per liter) with digestion time (hours) for loads corresponding to (A) 1.5, 2.0, 2.5, and 3.0 g of COD and (B) 3.5, 4.0, 4.5, and 5.0 g of COD.

Table 5. Values of the Apparent Kinetic Constants for Substrate (TOC) Consumption, S_{nb} , and S_b with Corresponding Standard Deviations for All Loadings

	load							
	1.5 g of COD	2.0 g of COD	2.5 g of COD	3.0 g of COD	3.5 g of COD	4.0 g of COD	4.5 g of COD	5.0 g of COD
S_{nb} (mg of TOC/L)	35 ± 10	30 ± 7	32 ± 17	54 ± 5	62 ± 24	95 ± 40	114 ± 25	76 ± 47
S_b (mg of TOC/L)	168 ± 15	223 ± 10	408 ± 31	478 ± 7	519 ± 29	696 ± 66	601 ± 38	782 ± 52
K_G' (h^{-1})	0.468 ± 0.101	0.335 ± 0.038	0.233 ± 0.048	0.223 ± 0.008	0.127 ± 0.020	0.162 ± 0.037	0.103 ± 0.016	0.046 ± 0.008

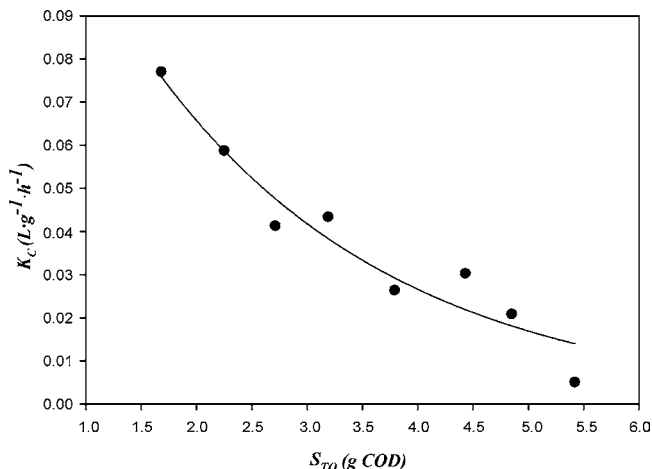


Figure 8. Variation in the specific kinetic constant for TOC consumption (K_C) as a function of initial substrate concentration, S_{T0} (grams of COD).

tional phase was less than the observed value for the wastewater feed (0.32), confirming the removal of volatile fatty acids initially present in the wastewater by the anaerobic biomass, and tendency of the system to come to operational stability.

Kinetics of Methane Production. Figure 2 shows the volume of methane accumulated (G) as a function of time for loads of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g of COD. The results show that the volume of methane produced increased with increasing load and that the time required for complete removal of the biodegradable fraction of each of the loads ranged between 48 and 72 h.

To characterize each experiment kinetically and thus facilitate comparisons, the following model was used (16–18):

$$G = G_m [1 - \exp(-K_G' t)] \quad (1)$$

G is the volume of methane accumulated (mL at standard temperature and pressure, STP) at a given time t (hours), G_m is the maximum methane volume accumulated at an infinite digestion time, and K_G' is an apparent kinetic constant for methane production (h^{-1}), which includes the biomass concentration

$$K_G' = K_G X \quad (2)$$

where K_G is the specific rate constant for methane production [$L/(g h)$] and X is the biomass concentration (g of VSS/L).

According to eq 1, methane production conforms to a first-order kinetic model (16, 19). As can be seen in Figure 2, G was zero at $t = 0$ and the rate of gas production became zero at $t = \infty$. As expected, the amount of gas produced increased with increasing load. The slopes of the curves decreased with increasing time: this decrease can be ascribed to the gradual decrease in the concentration of biodegradable substrate. Equation 1 shows good agreement with the experimental data. Thus, it seems reasonable to apply the proposed kinetic model to all of the loads studied in the reactor used.

The values of K_G' and G_m for each loading were calculated numerically from the experimental data by nonlinear regression using Sigma-Plot software (version 9.0). Table 3 shows the G_m and K_G' values obtained with their standard deviations. As can be seen, the standard deviations of G_m and K_G' values were less than 5 and 10%, respectively, for all of the loads studied, suggesting that the proposed model fits to the experimental data adequately.

Table 4 summarizes the variation in biomass concentration (grams of VSS per liter) with loading for all of the experiments carried out. As can be seen, the biomass concentration remained virtually constant throughout the experiments, which was as expected when the low microorganism yield coefficient (0.02–0.06 g of VSS/g COD) (7–9) and the loss of biomass as a consequence of the small volumes of sample taken from the reactor for effluent characterization were considered.

Once the biomass concentration values were determined, the values of the specific rate constant of methane production, K_G , were calculated using eq 2. These K_G values were plotted as a function of the load added (Figure 3). As can be seen in Figure 3, the specific rate constant, K_G , decreased markedly with substrate concentration in the reactor, showing the occurrence of an inhibition process. Specifically, the K_G value decreased 8.6 times when the load added increased from 1.5 to 5 g of COD. A similar behavior has been observed in the anaerobic digestion of wastewater produced in the manufacture of cellulose pulp from wheat straw (20), untreated molasses (21), and two-phase olive mill effluents (22) in batch reactors.

To evaluate the variations in experimental data, the theoretical values of maximum methane production (G_m) were calculated using eq 1 and plotted against their corresponding experimental values (Figure 4). These calculations were performed so as to give an error band of 5%. As can be seen, the deviations obtained were <5% in practically all cases, suggesting that the proposed model can be used to predict the behavior of this reactor very accurately and that the kinetic parameters obtained represent the activity of the microorganisms effecting the anaerobic digestion of this wastewater.

Methane Yield Coefficient and Biodegradability of the Wastewater. The yield coefficient of methane was determined from the experimental data on maximum methane volume produced (G_T) and the final and initial COD. By fitting (G_T , COD consumed) value pairs to a straight line (Figure 5), the methane yield coefficient coincides with the slope of the regression line and was found to be 295 mL of CH_4 at STP conditions/g of COD removed. This value agrees with the data reported in the literature for substrates that can easily be anaerobically biodegraded (9). The actual methane yield was 84% of the theoretical value of 0.35 L of methane/g of COD removed, ignoring any biomass growth and cell maintenance requirements (9). This clearly demonstrates the efficiency of the process at mesophilic temperature.

The high biodegradability of the wastewater can also be demonstrated by plotting the amount of substrate removed against substrate added for all of the experiments carried out.

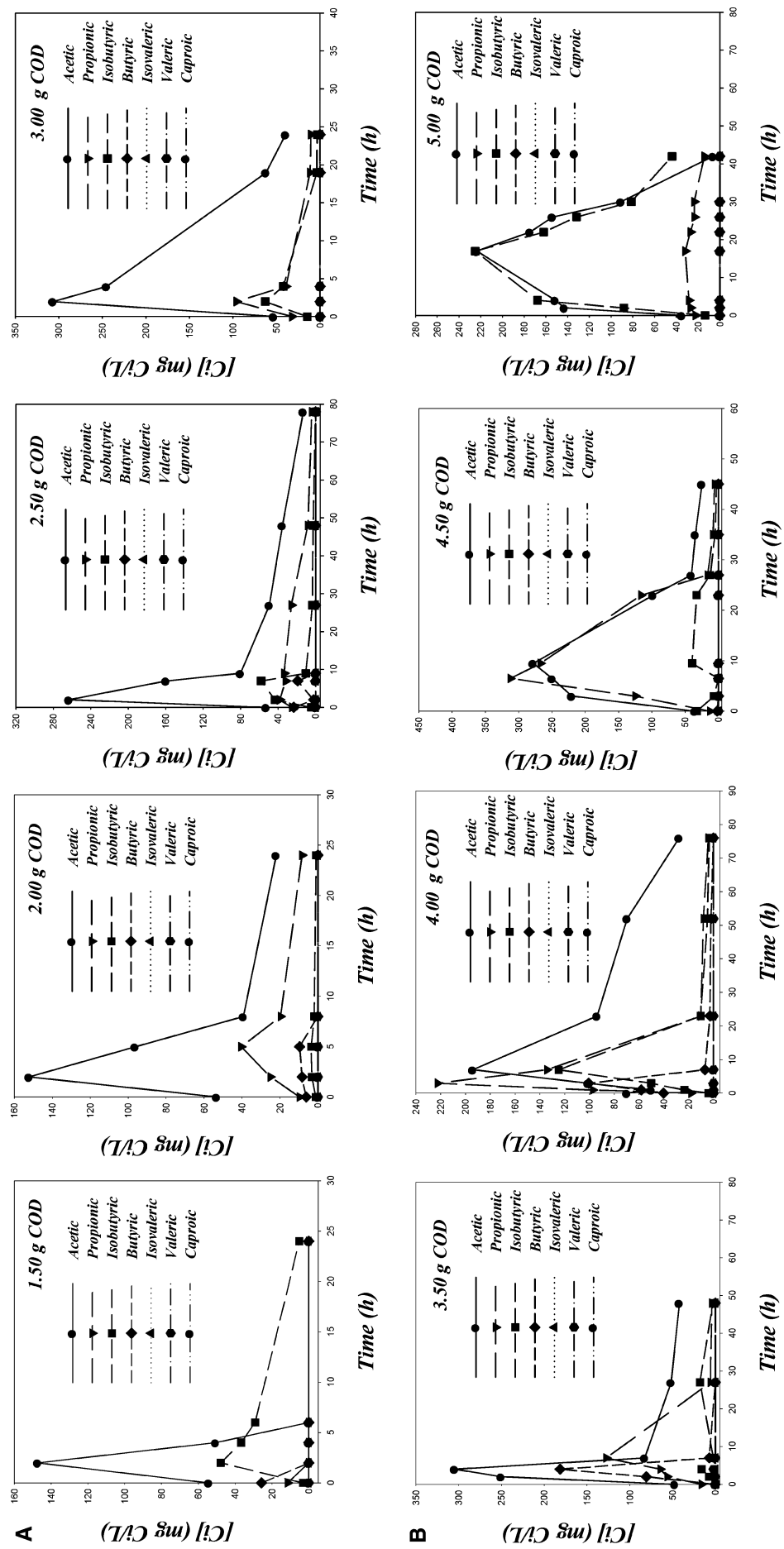


Figure 9. Variation in individual volatile fatty acids (acetic, C2, propionic, C3, butyric, C4, isobutyric, iC4, valeric, C5, isovaleric, iC5, and caproic, C6) with digestion time for loads of (A) 1.5, 2.0, 2.5, and 3.0 g of COD and (B) 3.5, 4.0, 4.5, and 5.0 g of COD.

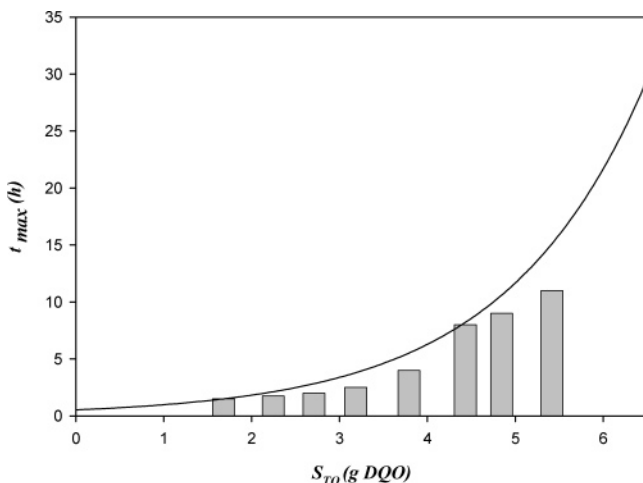


Figure 10. Maximum time necessary to achieve maximum VFA concentrations for each load (grams of COD).

Figure 6 shows these data in such a manner that the slope of the straight line obtained coincides with the percentage of the biodegradability of the waste, which was found to be 84%, a value that is maintained constant throughout the loads, although the substrate removal rate decreases with increasing loads as was previously demonstrated.

Finally, a COD balance around the reactor allows the following equation to be established (23):

$$\text{COD}_{\text{removed}} = \text{COD}_{\text{CH}_4} + \text{COD}_X + \text{COD}_{\text{cell metabolism}} \quad (3)$$

COD_{CH_4} represents the fraction of the initial COD transformed into methane, COD_X represents the fraction of COD used in the cell growth, and, finally, $\text{COD}_{\text{cell metabolism}}$ represents the fraction of COD used by microorganisms for their metabolism and maintenance. Taking into account that for this wastewater 84% of the COD is used for methane production, the remaining 16% is used for cell growth and metabolism. Therefore, most of the COD removed is employed for methane production and the microbial metabolism is very oriented toward gas generation.

Kinetics of Substrate Consumption. **Figure 7** shows the variation in total organic carbon (TOC in milligrams per liter) in the reactor with digestion time (hours) for loads corresponding to 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g of COD. As can be seen, the TOC decreases gradually with time until reaching 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 cessation in methane production.

In accordance with these results, the following equation is proposed:

$$S_t = S_{nb} + (S_0 - S_{nb}) \exp(-K_C' t) \quad (4)$$

S_t is the effluent substrate concentration (mg of TOC/L) at a given digestion time, t (h); S_0 is the initial substrate concentration (mg of TOC/L); S_{nb} is the non-biodegradable substrate concentration (mg of TOC/L); and K_C' is an apparent kinetic constant that includes the biomass concentration (X). Therefore

$$K_C' = K_C X \quad (5)$$

allowing determination of the kinetic constant for each load studied. Moreover, the difference ($S_0 - S_{nb}$) can be defined as the biodegradable substrate present in the wastewater.

By nonlinear regression using the SigmaPlot software (version 9.0) all of the kinetic parameters related to eq 4 were obtained. **Table 5** summarizes the values of the apparent kinetic constant, K_C' , as well as the S_{nb} and S_b values with their corresponding standard deviations for all of the loads studied. The low standard deviation values for all of these parameters show an adequate fitting of the experimental data to the proposed model. As can be calculated from **Table 5** the total substrate concentration ($S_{nb} + S_b$) is directly proportional to the initial substrate concentration present in the reactor, expressed in COD. This again indicates that the kinetic model proposed gives an adequate fit to the experimental data obtained.

As can be seen in **Table 5**, the apparent kinetic constant decreases with increasing loads. Once the biomass values were known (**Table 4**), the values of the specific kinetic constant for TOC consumption (K_C) were calculated by using eq 5.

Figure 8 illustrates the variation in the specific kinetic constant for TOC consumption (K_C) as a function of the initial substrate concentration, S_{TO} (grams of COD per liter). A marked decrease in K_C values with increasing load applied was observed, again suggesting the occurrence of an inhibition process.

Another way to study the biodegradability of the wastewater is through the conversion of the soluble organic matter, which can be defined by the following equation:

$$Z = [(S_{t0} - S_t)/S_{t0}] \times 100 \quad (6)$$

Z is the conversion (%), S_{t0} is the initial substrate (mg of TOC), and S_t is the substrate (mg of TOC) at a given time t . It was confirmed that the Z values fitted a decreasing exponential model similar to that given by eq 1:

$$Z = Z_m [1 - \exp(-K' t)] \quad (7)$$

Z_m is the overall conversion for each experiment and K' is a parameter related to the rate at which that conversion is reached. From the experimental data, a mean value for Z_m of 88.2% was obtained, again confirming the high biodegradability of the wastewater.

Evolution of the Volatile Fatty Acids with Time. **Figure 9** shows the variation in individual volatile fatty acids (acetic, C2; propionic, C3; butyric, C4; isobutyric, iC4; valeric, C5; isovaleric, iC5; and caproic, C6) with digestion time for loads of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g of COD, respectively. As can be seen, the main volatile fatty acids generated in all of the loads studied were acetic and propionic, which facilitated conversion into methane by the anaerobic microorganisms involved in the process. From the data plotted in **Figure 9**, the global concentrations of volatile fatty acids (VFA) (expressed as acetic acid) were calculated for each load considered.

Figure 10 plots the maximum time necessary to achieve the maximum concentrations of VFA for each load added to the reactor. As can be seen, the maximum time for reaching the maximum VFA concentration increased exponentially when the load increased from 1.5 to 5.0 g of COD, which again demonstrates the inhibition of the process by increasing substrate concentration.

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