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# Kinetic modelling of the anaerobic digestion of wastewater derived from the pressing of orange rind produced in orange juice manufacturing

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**Abstract**

A simplified kinetic model for studying the anaerobic digestion of wastewater derived from the pressing of orange rind as a result of orange juice production was proposed on the basis of the experimental results obtained. The process was conducted in a laboratory-scale completely stirred tank reactor operating in batch mode at mesophilic temperature (35 °C), with COD loads in the range of 2–5 g COD. The following simplified three-step reaction scheme was proposed: (1) hydrolysis and conversion of complex organic compounds into intermediate products of lower molecular weight; (2) conversion of these intermediates to volatile fatty acids (VFA); and (3) methanization of the VFA by methanogenic microorganisms. A mathematical model based on four segregated differential equations was formulated assuming that a fraction of this substrate is non-biodegradable and the above-mentioned steps follow first-order kinetics. It was found that the kinetic constants corresponding to these three stages ($K_0$, $K_1$ and $K_2$) decreased markedly with the load added to the reactor, showing the occurrence of an inhibition process. In addition, it was observed that the methanogenic step was the slowest in the overall anaerobic process. Finally, the model was validated by comparing the theoretical curves obtained with the corresponding experimental data of organic matter, VFA and methane. The deviations obtained (less than 20%) in most cases demonstrated the suitability of the mathematical model proposed and suggested that the parameters obtained represent and predict the activity of the microorganisms involved in the anaerobic digestion process of this wastewater.


*Keywords:* Kinetic modelling; Anaerobic digestion; Wastewater from pressing of orange rind; Batch reactor; Mesophilic temperature

## 1. Introduction

Spain is one of the largest orange producers in the world [1–3]. Orange juice is a by-product of oranges [3]. In the process of orange juice production a large volume of orange rind is generated, which in turn is used as a raw material in the manufacture of some cattle feeds. The first step in this process is the pressing of the rind. In the pressing process calcium hydroxide is used as binder, and significant amounts of wastewater are intermittently generated. These are heavy pollutants due to the high concentration of organic matter (150 g COD/L) and alkalinity [4].

Anaerobic digestion is now increasingly being used to treat organic effluents, particularly for wastewaters containing medium to high levels of biodegradable organic matter. Due to the high organic load of wastewater derived from the pressing of orange rind produced during the manufacture of orange juice, anaerobic digestion is suitable for this kind of wastewater [4]. Anaerobic digestion may be defined as the biological conversion of organic material to a variety of end products including 'biogas' whose main constituents are methane (65–70%) and carbon dioxide [5–8]. The advantages of anaerobic digestion include low levels of biological sludge, high efficiency and the production of methane which can be used as an energy source for on site heating and electricity. In contrast to aerobic wastewater treatment processes, which are heavy fossil fuel utilizers, anaerobic digestion processes result in a net reduction in $CO_2$ emissions. Another advantage of anaerobic digestion is that a digester can be started up after more than 8 months under non-feeding conditions [8], and is thus suitable for the treatment of seasonal wastes.

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The anaerobic conversion process of a waste to methane gas involves several biological reaction steps, some authors propose only two stages [9], but there are other researchers that propose from three to nine stages in the process [9]. Most authors have considered three main stages [7–9]: (1) the complex biopolymers are hydrolytically converted to low-molecular-weight compounds able to be used as substrates by cells; (2) the hydrolysed waste is converted to volatile organic acids by an anaerobic microbiota; and (3) finally, methane is produced from volatile organic acids by methanogenic microorganisms [8,9].

Process modelling is a useful tool for describing and predicting the performance of anaerobic digestion systems. Monod type kinetic models have been widely used to describe the process kinetics of anaerobic digesters [10,11]. Although there has been some success in applying Monod type kinetics to the anaerobic process some researchers found it difficult to apply them for their systems [10,11]. For instance, it has been shown [11] that the effluent substrate concentration, expressed as COD, was not independent of the substrate concentration entering the reactor when pure or heterogeneous cultures were used. In the equation proposed by Contois [11], the specific growth rate was considered as a function of the growth-limiting nutrient in both input and effluent substrate concentration by using an empirical constant, which was related to microbial concentration. On this basis, Chen and Hashimoto developed kinetic models for substrate utilization and methane production and suggested that the Contois type kinetic models would be more suitable than the Monod type kinetic models to predict digester performance [8,10,11].

Multiculture system kinetics may be desirable in view of the heterogeneous nature of the microbial population performing the various bioconversion steps involved. However, the kinetic models based on this premise necessarily involve a number of kinetic equations and coefficients making them highly complex, as shown by the reported models [10,11]. Complexity does not necessarily equate to accuracy and there is still a strong case in favour of a simpler kinetic treatment based on a single culture system. Methanogenesis is particularly suited to this approach as there is a strong holistic characteristics in the process. Various cultures and bioconversion steps in digestion are interdependent and the whole process has certain self-regulatory characteristics within the process limits [12].

Mosey [13] and Kalyuzhnyi and Davlyatshina [14] developed mathematical models describing the kinetics of acidogenesis, acetogenesis, acetoclastic methanogenesis, hydrogenothrophic methanogenesis, bacterial decay, pH and various inhibitions of the mentioned steps. Mösche and Jördening [15] also studied the acetate and propionate degradation and inhibition. Cuevas et al. [16] developed a kinetic model to describe the different steps of substrate degradation in anaerobic sequencing batch reactors (ASBRs). Hu et al. [17] investigated the anaerobic digestion kinetics of ice-cream wastewater using Monod and Contois models. Both kinetic models were evaluated with a set of routine analytical data obtained from a pilot-scale (5 $m^3$) anaerobic contact digester applied to a similar wastewater. The Contois equation was more suitable than the Monod equation for describing the process kinetics. Aguilar et al. [18] studied the kinetic parameters of total volatile acids (TVA) degradation for two methanogenic populations previously enriched in continuous digesters feed with acetate or glucose as the main carbon source. Progress curves for TVA utilization were used to calculate $K_s$ and $\mu_{max}$, and comparison between results obtained for both inocula was made using substrate affinities for acetic, propionic, *n*-butyric and *i*-butyric acids. Lokshina et al. [19] used the integrated Monod and Haldane models to evaluate the kinetic coefficients using the methane accumulation curves of low-temperature acetoclastic methanogenesis. Samples of lake sediments and biomass taken from a low-temperature (UASB) reactor were used as inoculum in batch assays for acetate methanation. In comparison, the Monod and Haldane models were applied to evaluate the kinetic coefficients for mesophilic acetoclastic methanogenesis accomplished by the pure culture of *Methanosarcina barkeri* strain MS. For the wide range of initial acetate concentrations applied to the UASB biomass, a better fit was obtained using the Haldane models and their exponential approximations. García-Ochoa et al. [9] used first- and second-order models to describe the anaerobic digestion of livestock manure. The results obtained showed that the second model had both statistical and physical meanings in the parameter values obtained. The model took into account a simplified reaction scheme formed by six reactions. Several simplifications were made yielding four key compounds to be analysed and fitted to the model as production-rate expressions (total biomass, COD, VFA, and methane). Three main stages were considered in the process: enzymatic hydrolysis, growth of acetogenic microorganisms and growth of methanogenic microorganisms.

Therefore, and as was widely reported in the literature, anaerobic digestion consists of a multitude of biochemical reactions in series and in parallel that occur simultaneously. Due to the complexity of the biochemical reactions and the presence of possible inhibitors or delayer compounds in the wastewater derived from the pressing of orange rind produced in orange juice manufacturing that is subjected to anaerobic treatment, a more detailed study of the kinetics is necessary for understanding and optimising the process. As a consequence of these considerations, the aim of this work was to develop a simplified kinetic model for anaerobic digestion of the wastewater derived from the pressing of orange rind, which could describe the evolution of the organic matter (total organic carbon), volatile fatty acids (VFA) and methane production with digestion time. The model takes into account that complex organic matter is previously hydrolysed and transformed into simpler low-molecular-weight compounds (intermediate substances), which are further transformed into VFA, and finally the VFA resulting from the decomposition of the intermediate substances are transformed to methane. The calculation of the parameters of the model will be made by fitting of experimental data of anaerobic digestion of the above-mentioned substrate to a set of segregated differential equations. The study was carried out in batch mode in a laboratory-scale completely stirred tank reactor at mesophilic temperature (35 °C).

## 2. Materials and methods

### 2.1. Equipment

The reactor used for the anaerobic digestion of wastewater derived in the pressing of orange rind generated in orange juice production consisted of a 1-L Pyrex flask with four connections: 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 at 35 °C 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 $CO_2$ produced, a tightly closed bubbler containing a NaOH solution (6N) was connected between the two elements. The methane displaced a measurable quantity of water from the reservoir equivalent to the volume of methane produced. In order to guarantee that all the $CO_2$ from the biogas was removed, the NaOH solution was deleted and substituted by a new fresh solution every week.

### 2.2. Inoculum

The reactor was inoculated with methanogenically active granular 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 COD/(g VSS day) [20]. The contents of total suspended solids (TSS) and volatile suspended solids (VSS) in the inoculum were: 46.6 and 38.9 g/L, respectively.

### 2.3. Wastewater

Table 1 shows the characteristics of the raw wastewater derived from the pressing of orange rind 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 aluminium sulphate as flocculant (at a concentration of 100 mg/L) and to pH reduction using a solution of sulphuric acid. Finally, nitrogen (as $NH_4Cl$) and phosphorus (as $KH_2PO_4$) 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 also shows the characteristics of this pre-treated wastewater used as substrate in the anaerobic digestion experiments.

### 2.4. Experimental procedure

The anaerobic reactor was initially loaded with the above-described inoculum (7 g 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 [8,21]. The compositions of the nutrient and trace element solutions are given in detail elsewhere [4].

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 COD over a 10-day period. Finally, after this previous stage, and with the objective of acclimatizing the biomass to the substrate, before the beginning the experiments the reactor was fed with four loads of 1 g 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 total duration of the start-up and acclimatization stages was around 30 days. 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 pre-

Table 1
Composition and characteristics of raw wastewater derived from the pressing of orange rind and of wastewater treated with aluminium sulphate to remove part of its total and suspended solids contents and slightly acidified with sulphuric acid prior to the addition of N and P[a] (mean values ± standard deviations)

| Parameter | Raw wastewater | Pre-treated wastewater used as substrate |
|---|---|---|
| pH | 11.2 ± 0.5 | 5.5 ± 0.3 |
| Alkalinity (mg $CaCO_3$/L) | 8360 ± 410 | 1550 ± 75 |
| Volatile acidity, VA (mg acetic acid/L) | 695 ± 30 | 600 ± 25 |
| Total chemical oxygen demand, COD (mg/L) | 147,680 ± 7320 | 130,040 ± 6500 |
| Soluble COD, $COD_s$ (mg/L) | 140,300 ± 6950 | 128,740 ± 6410 |
| Total organic carbon, TOC (mg/L) | 52,970 ± 2600 | 47,395 ± 2340 |
| Total suspended solids, TSS (mg/L) | 20,780 ± 1030 | 17,030 ± 850 |
| Mineral suspended solids, MSS (mg/L) | 3200 ± 150 | 1230 ± 50 |
| Volatile suspended solids, VSS (mg/L) | 17,580 ± 860 | 15,800 ± 750 |
| Total solids, TS (mg/L) | 151,900 ± 7550 | 138,025 ± 6900 |
| Mineral solids, MS (mg/L) | 14,160 ± 705 | 14,225 ± 710 |
| Volatile solids, VS (mg/L) | 137,740 ± 6800 | 123,800 ± 6120 |
| $PO_4^{3-}$ (mg/L) | – | 2.6 ± 0.1 |
| $N–NO_3^-$ (mg/L) | – | 7.2 ± 0.3 |
| $N–NH_4^+$ (mg/L) | – | 56.7 ± 2.8 |

[a] Values are averages of four determinations on four samples.

treated wastewater as substrate. During the experiments, the organic load added to the reactor was gradually increased from 2.0 to 2.5, 3.0, 3.5, 4.0, 4.5 and to 5.0 g COD. In all cases, the volume of methane was measured as a function of time and the initial and final COD, TOC, VSS, pH, volatile acidity, and alkalinity 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.

### 2.5. Chemical analyses

The following parameters were determined in the effluent at each loading: pH, $COD_s$, TOC, VSS, volatile acidity (VA) and alkalinity. All analyses were carried out in accordance to Standard Methods [22].

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

Separate volatile fatty acids (acetic, propionic, butyric, *iso*-butyric, valeric, *iso*-valeric and caproic acids) were determined using a gas chromatograph Hewlett-Packard HP-5890 equipped with a 15 m × 0.53 mm (I.D.) Nukol-silica semi-capillary 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.

## 3. Results and discussion

### 3.1. Chemical parameters and biodegradability

Figs. 1–7 show the evolution of the organic matter concentration (OM), of the volatile fatty acids and of the volume of methane accumulated (all expressed as mg C/L) as a function of the digestion time for the loads added of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 g COD, respectively. As can be seen in all cases, the organic matter concentration (mg C/L) decreases gradually with time until it reaches an asymptotic value (which is not equal to zero) as a consequence of the occurrence of compounds which are not anaerobically biodegradable. This coincides with the cessation in methane production. Simultaneously, it can be observed that the volume of methane produced rose as the load was increased and that the time required for complete removal of the biodegradable fraction of each load ranged between 48 and 72 h. An exponential relationship between cumulative methane production and digestion time was also observed, as was previously reported [4].

As reported in a preliminary study [4] under the operational conditions used in this investigation the pH in the reactor remained approximately constant at all the applied loadings, with a mean value of 7.5 (within the optimal range for methanogens [8,21]), and with extreme values of 7.3 and 7.8. The buffering capacity of the experimental system was maintained at favourable levels with excessive total alkalinity present at all loadings, at a mean value of 3220 mg $CaCO_3$/L. This buffering protects against the possible acidification of the reactor, giving a pH of the same order as the optimal for methanogenic microorganisms [21]. This previous study also

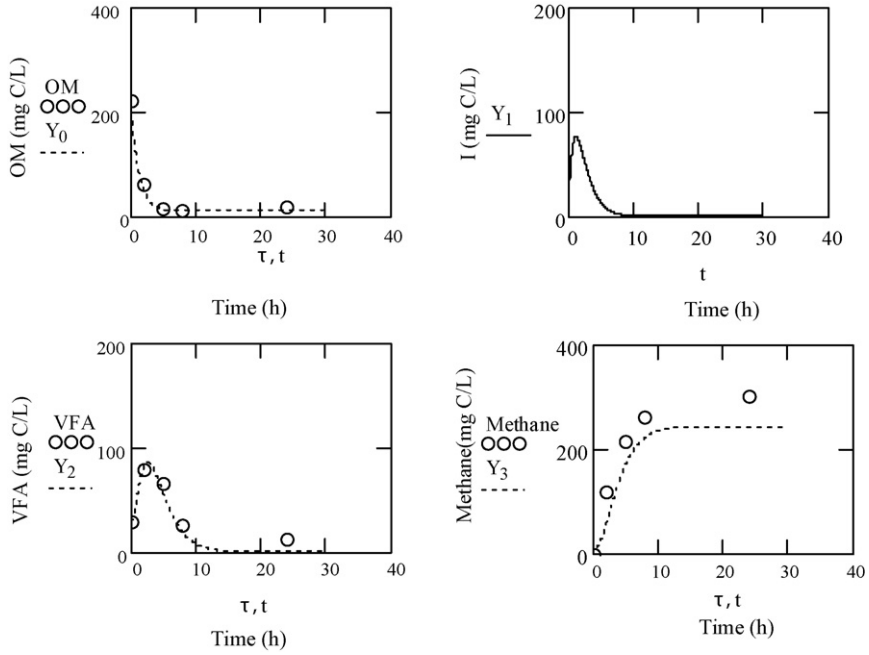


Fig. 1. Variation of the experimental (◯) and theoretical (solid curves) organic matter (OM), volatile fatty acids (VFA), intermediate products (*I*) concentrations and methane as predicted by the model with the digestion time for the load of 2.0 g COD.

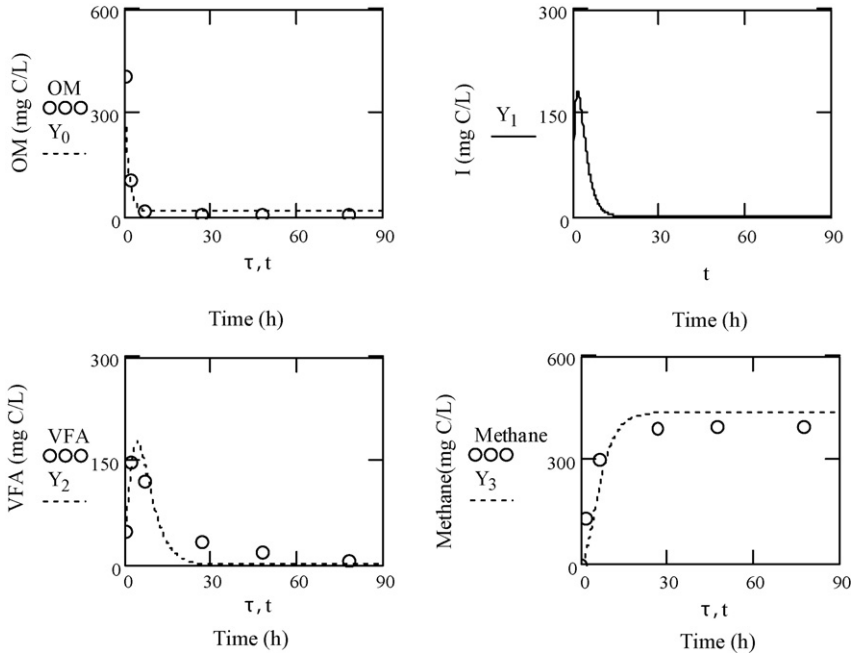


Fig. 2. Variation of the experimental (◯) and theoretical (solid curves) organic matter (OM), volatile fatty acids (VFA), intermediate products (*I*) concentrations and methane as predicted by the model with the digestion time for the load of 2.5 g COD.

reported that the methane yield coefficient obtained for these operational conditions was found to be 295 mL $CH_4$ at STP conditions per gram of COD removed. This value is in accordance with the data reported in the literature for substrates that can easily be anaerobically biodegraded [8]. This actual methane yield was 84% of the theoretical value of 0.35 L methane/g COD removed, ignoring any biomass growth and cell maintenance requirements [8]. This fact clearly demonstrated the efficiency of the anaerobic process at mesophilic temperature. In addition, the volatile fatty acids/alkalinity ratio obtained in this study was always lower than the suggested limits for digester failure (0.3–0.4) [21,23] for all the loadings used (2.0–5.0 g COD), indicating the stability of the reactor.

Finally, Tables 2–8 show the variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and

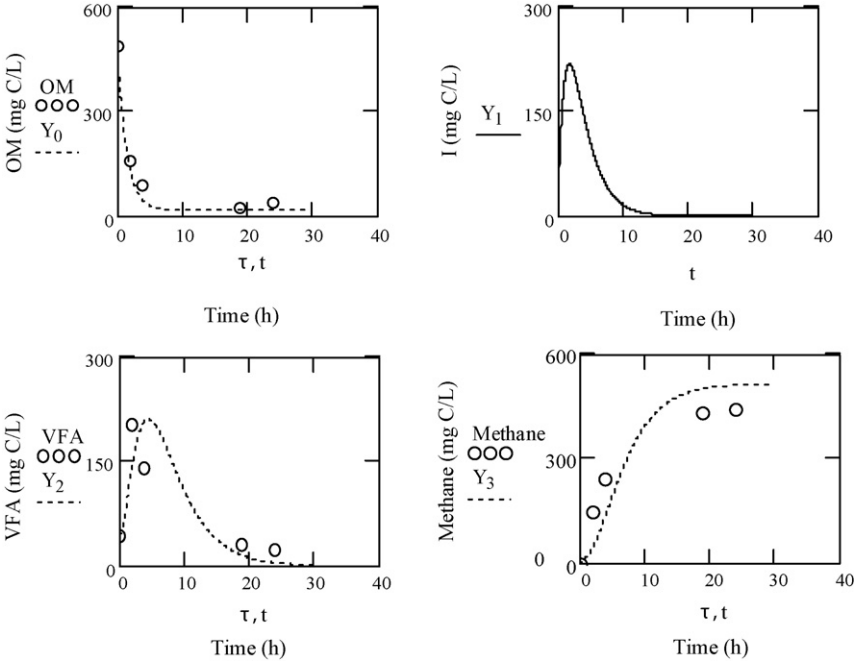


Fig. 3. Variation of the experimental (◯) and theoretical (solid curves) organic matter (OM), volatile fatty acids (VFA), intermediate products (*I*) concentrations and methane as predicted by the model with the digestion time for the load of 3.0 g COD.

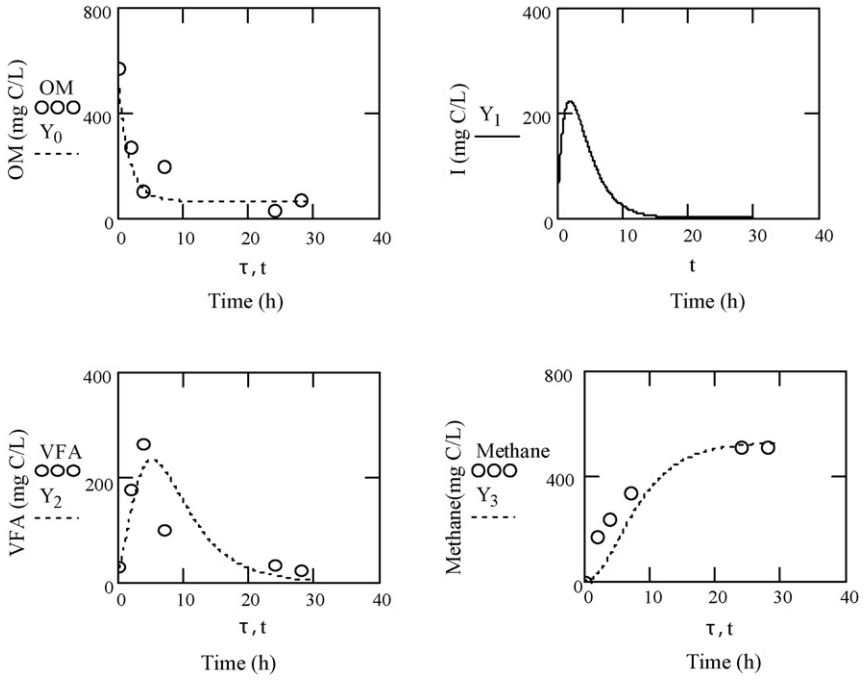


Fig. 4. Variation of the experimental (◯) and theoretical (solid curves) organic matter (OM), volatile fatty acids (VFA), intermediate products (*I*) concentrations and methane as predicted by the model with the digestion time for the load of 3.5 g COD.

total volatile fatty acid concentration (expressed as mg C/L) with digestion time for the loads of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 g COD, respectively. As can be seen, the main volatile fatty acids generated in all the loads studied were acetic and propionic, which facilitated conversion into methane by the anaerobic microorganisms involved in the process. It can also be observed that for loads in the range of 2.0–4.0 g COD, total acidity and VFA (expressed as mg acetic acid/L and mg C/L, respectively) increased considerably with time during the first 2–4 h of digestion after which a marked decrease was observed. However, for the loads of 4.5 and 5.0 g COD, the time necessary for achieving the maximum VFA production was 9.5 and 17.0 h, respectively, which demonstrates the inhibition of the process by increasing substrate concentration.

In the following sections the same reactor was used under the same operating conditions to formulate and assess a

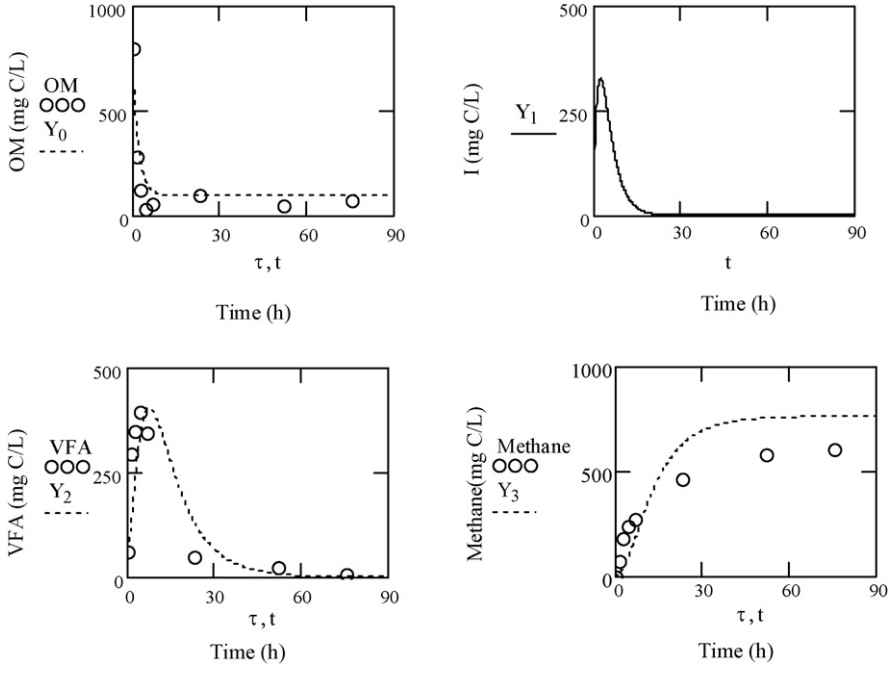


Fig. 5. Variation of the experimental (◯) and theoretical (solid curves) organic matter (OM), volatile fatty acids (VFA), intermediate products (*I*) concentrations and methane as predicted by the model with the digestion time for the load of 4.0 g COD.

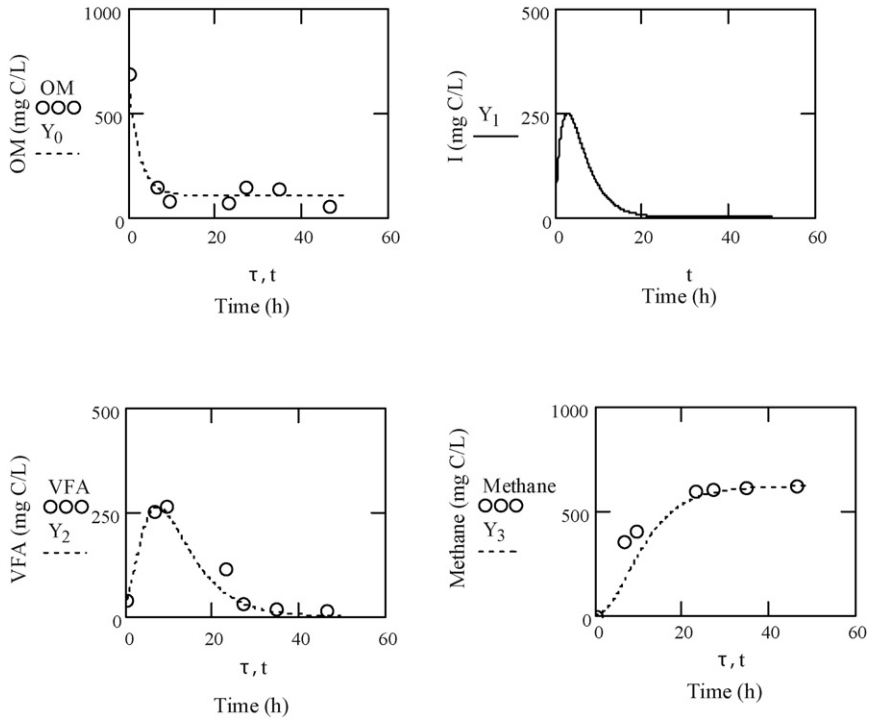


Fig. 6. Variation of the experimental (◯) and theoretical (solid curves) organic matter (OM), volatile fatty acids (VFA), intermediate products (*I*) concentrations and methane as predicted by the model with the digestion time for the load of 4.5 g COD.

kinetic model for predicting the behaviour of the reactor and for validating the evolution of the organic matter content, together with VFA production and consumption and methane generation.

### 3.2. Kinetic modelling

With the aim of formulating a kinetic model for anaerobic digestion of wastewater derived from the pressing of orange

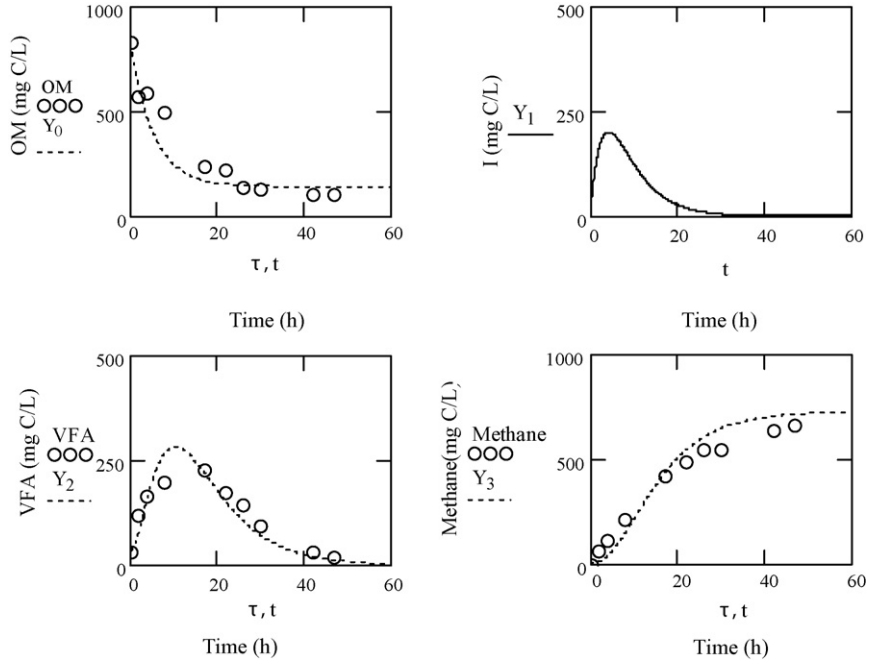


Fig. 7. Variation of the experimental (◯) and theoretical (solid curves) organic matter (OM), volatile fatty acids (VFA), intermediate products (*I*) concentrations and methane as predicted by the model with the digestion time for the load of 5.0 g COD.

Table 2
Variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and total volatile fatty acid concentration (VFA, expressed as mg C/L) with digestion time for the load of 2.00 g COD

| Time (h) | Acetic acid (mg/L) | Propionic acid (mg/L) | Isobutyric acid (mg/L) | Butyric acid (mg/L) | Isovaleric acid (mg/L) | Valeric acid (mg/L) | Total acidity (mg acetic acid/L) | VFA (mg C/L) |
|---|---|---|---|---|---|---|---|---|
| 0 | 53.3 | 9.7 | 1.1 | 6.1 | 0.0 | 0.0 | 74.9 | 30.0 |
| 2 | 152.2 | 25.0 | 3.1 | 8.3 | 0.0 | 0.0 | 198.1 | 79.2 |
| 5 | 96.3 | 40.4 | 3.5 | 9.6 | 0.0 | 0.0 | 163.2 | 65.3 |
| 8 | 39.2 | 19.6 | 1.9 | 0.0 | 0.0 | 0.0 | 65.7 | 26.3 |
| 24 | 21.9 | 8.3 | 0.9 | 0.0 | 0.0 | 0.0 | 33.3 | 13.3 |

Table 3
Variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and total volatile fatty acid concentration (VFA, expressed as mg C/L) with digestion time for the load of 2.50 g COD

| Time (h) | Acetic acid (mg/L) | Propionic acid (mg/L) | Isobutyric acid (mg/L) | Butyric acid (mg/L) | Isovaleric acid (mg/L) | Valeric acid (mg/L) | Total acidity (mg acetic acid/L) | VFA (mg C/L) |
|---|---|---|---|---|---|---|---|---|
| 0 | 52.6 | 25.2 | 4.6 | 23.1 | 0.0 | 0.0 | 120.9 | 48.4 |
| 2 | 263.3 | 38.2 | 43.2 | 2.0 | 0.0 | 0.0 | 371.3 | 148.5 |
| 7 | 159.4 | 31.6 | 58.0 | 19.1 | 0.0 | 0.0 | 303.0 | 121.2 |
| 9 | 80.0 | 33.6 | 10.3 | 0.0 | 0.0 | 0.0 | 135.0 | 54.0 |
| 27 | 49.1 | 25.7 | 3.2 | 0.0 | 0.0 | 0.0 | 84.7 | 33.9 |
| 48 | 35.3 | 8.0 | 2.6 | 0.0 | 0.0 | 0.0 | 48.7 | 19.5 |
| 78 | 13.1 | 3.8 | 0.0 | 0.0 | 0.0 | 0.0 | 17.6 | 7.1 |

Table 4
Variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and total volatile fatty acid concentration (VFA, expressed as mg C/L) with digestion time for the load of 3.00 g COD

| Time (h) | Acetic acid (mg/L) | Propionic acid (mg/L) | Isobutyric acid (mg/L) | Butyric acid (mg/L) | Isovaleric acid (mg/L) | Valeric acid (mg/L) | Total acidity (mg acetic acid/L) | VFA (mg C/L) |
|---|---|---|---|---|---|---|---|---|
| 0 | 53.3 | 32.3 | 14.7 | 0.0 | 0.0 | 0.0 | 112.5 | 45.0 |
| 2 | 306.5 | 95.9 | 63.0 | 0.0 | 0.0 | 0.0 | 509.1 | 203.7 |
| 4 | 245.0 | 39.1 | 42.2 | 0.0 | 0.0 | 0.0 | 350.1 | 140.1 |
| 19 | 62.1 | 11.5 | 3.7 | 0.0 | 0.0 | 0.0 | 81.1 | 32.4 |
| 24 | 39.4 | 10.4 | 3.2 | 0.0 | 0.0 | 0.0 | 56.4 | 22.6 |

rind, the following considerations were taken into account:

- The anaerobic conversion process of a complex waste material involves three main biological reaction steps: (a) the complex or high-molecular-weight compounds contained in the substrate are hydrolysed and converted into intermediate products of lower molecular weight (such as aminoacids, sugars, alcohols, organic acids, etc.), compounds that can be used as substrates by cells; (b) these intermediate products are converted into volatile fatty acids; (c) and finally, the VFA are transformed into methane by methanogenic microorganisms. These three steps can be summarized as follows:

$$\text{Organic matter (OM)} \xrightarrow{K_0} \text{intermediate products } (I) \xrightarrow{K_1} \text{VFA} \xrightarrow{K_2} \text{methane}$$

where $I$ is the concentration of intermediate products (expressed as C), VFA is the concentration of volatile fatty acids (expressed as C), $K_0$ is the kinetic constant for organic matter (expressed as carbon) degradation, $K_1$ is the kinetic

Table 5
Variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and total volatile fatty acid concentration (VFA, expressed as mg C/L) with digestion time for the load of 3.50 g COD

| Time (h) | Acetic acid (mg/L) | Propionic acid (mg/L) | Isobutyric acid (mg/L) | Butyric acid (mg/L) | Isovaleric acid (mg/L) | Valeric acid (mg/L) | Total acidity (mg acetic acid/L) | VFA (mg C/L) |
|---|---|---|---|---|---|---|---|---|
| 0 | 47.3 | 16.3 | 0.0 | 0.3 | 0.0 | 0.0 | 67.4 | 27.0 |
| 2 | 250.2 | 56.2 | 7.6 | 80.7 | 0.0 | 0.0 | 439.0 | 175.6 |
| 4 | 304.6 | 64.2 | 16.0 | 181.4 | 0.0 | 2.4 | 655.3 | 262.1 |
| 7 | 82.4 | 127.5 | 2.1 | 7.0 | 0.0 | 0.0 | 249.9 | 100.0 |
| 27 | 51.6 | 5.5 | 18.0 | 0.0 | 0.0 | 0.0 | 82.8 | 33.1 |
| 48 | 42.0 | 4.0 | 2.6 | 0.0 | 0.0 | 0.0 | 50.5 | 20.2 |

Table 6
Variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and total volatile fatty acid concentration (VFA, expressed as mg C/L) with digestion time for the load of 4.00 g COD

| Time (h) | Acetic acid (mg/L) | Propionic acid (mg/L) | Isobutyric acid (mg/L) | Butyric acid (mg/L) | Isovaleric acid (mg/L) | Valeric acid (mg/L) | Total acidity (mg acetic acid/L) | VFA (mg C/L) |
|---|---|---|---|---|---|---|---|---|
| 0 | 69.7 | 17.9 | 4.0 | 40.2 | 0.0 | 0.0 | 151.9 | 60.7 |
| 1 | 50.0 | 97.4 | 23.4 | 58.1 | 0.0 | 0.0 | 279.6 | 111.9 |
| 3 | 101.1 | 222.6 | 50.2 | 100.6 | 0.0 | 0.0 | 577.5 | 231.0 |
| 7 | 194.0 | 134.2 | 124.7 | 6.8 | 0.0 | 0.0 | 536.6 | 214.6 |
| 23 | 93.8 | 10.1 | 10.3 | 2.8 | 0.0 | 0.0 | 123.9 | 49.6 |
| 52 | 69.6 | 8.0 | 4.8 | 2.0 | 0.0 | 0.0 | 88.5 | 35.4 |
| 72 | 27.7 | 4.0 | 3.4 | 0.0 | 0.0 | 0.0 | 37.2 | 14.9 |

Table 7
Variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and total volatile fatty acid concentration (VFA, expressed as mg C/L) with digestion time for the load of 4.50 g COD

| Time (h) | Acetic acid (mg/L) | Propionic acid (mg/L) | Isobutyric acid (mg/L) | Butyric acid (mg/L) | Isovaleric acid (mg/L) | Valeric acid (mg/L) | Total acidity (mg acetic acid/L) | VFA (mg C/L) |
|---|---|---|---|---|---|---|---|---|
| 0.0 | 35.3 | 12.7 | 32.3 | 2.0 | 0.0 | 0.0 | 97.5 | 39.0 |
| 3.0 | 220.2 | 124.6 | 6.6 | 0.0 | 0.0 | 0.0 | 380.7 | 152.3 |
| 6.5 | 249.4 | 312.4 | 0.0 | 0.0 | 0.0 | 0.0 | 629.4 | 251.7 |
| 9.5 | 277.8 | 267.2 | 39.4 | 0.0 | 0.0 | 0.0 | 656.5 | 262.6 |
| 23.0 | 98.1 | 116.1 | 32.7 | 0.0 | 0.0 | 0.0 | 283.9 | 113.6 |
| 27.0 | 40.5 | 16.2 | 12.2 | 0.0 | 0.0 | 0.0 | 76.8 | 30.7 |
| 35.0 | 34.3 | 8.0 | 4.9 | 0.0 | 0.0 | 0.0 | 50.8 | 20.3 |
| 45.0 | 24.6 | 5.6 | 2.9 | 0.0 | 0.0 | 0.0 | 35.4 | 14.2 |

constant for intermediate products (expressed as carbon) removal and $K_2$ is the kinetic constant for VFA (expressed as C) degradation and conversion to methane.

- It was also assumed that this wastewater is partially biodegradable. Therefore, the total organic matter concentration is the sum of the biodegradable ([OM]b) and non-biodegradable ([OM]nb) concentrations. Consequently, the fraction of biodegradable organic matter is the difference between the total and non-biodegradable organic matter concentrations ([OM]b = [OM] − [OM]nb).
- The three afore-mentioned steps follow a first-order kinetics.
- All concentrations of OM, $I$ and VFA including methane production were expressed as Carbon.

Taking these considerations and the above simplified reaction scheme into account, the following kinetic model can be established using the differential equations as follows:

$$\frac{-\mathrm{d[OM]}}{\mathrm{d}t} = K_0\{[\mathrm{OM}] - [\mathrm{OM}]_{\mathrm{nb}}\} \quad (1)$$

$$\frac{\mathrm{d}[I]}{\mathrm{d}t} = K_0\{[\mathrm{OM}] - [\mathrm{OM}]_{\mathrm{nb}}\} - K_1[I] \quad (2)$$

$$\frac{\mathrm{d[VFA]}}{\mathrm{d}t} = K_1[I] - K_2[\mathrm{VFA}] \quad (3)$$

$$\frac{\mathrm{d}[\mathrm{CH_4}]}{\mathrm{d}t} = K_2[\mathrm{VFA}] \quad (4)$$

Eq. (1) describes the organic matter consumption as a function of the biodegradable organic matter concentration, while Eqs. (2) and (3) describe the evolution of intermediate products and VFA, respectively, with time. Finally, Eq. (4) relates the methane production with the VFA concentration. Given that the biomass concentration remained virtually constant throughout

Table 8
Variation of the individual volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric), total acidity (expressed as mg acetic acid/L) and total volatile fatty acid concentration (VFA, expressed as mg C/L) with digestion time for the load of 5.00 g COD

| Time (h) | Acetic acid (mg/L) | Propionic acid (mg/L) | Isobutyric acid (mg/L) | Butyric acid (mg/L) | Isovaleric acid (mg/L) | Valeric acid (mg/L) | Total acidity (mg acetic acid/L) | VFA (mg C/L) |
|---|---|---|---|---|---|---|---|---|
| 0 | 34.9 | 22.4 | 13.6 | 0.0 | 0.0 | 0.0 | 80.7 | 32.3 |
| 2 | 143.0 | 26.8 | 88.2 | 0.0 | 0.0 | 0.0 | 295.9 | 118.4 |
| 4 | 151.4 | 28.4 | 167.7 | 0.0 | 0.0 | 0.0 | 414.6 | 165.8 |
| 17 | 223.7 | 31.9 | 225.1 | 0.0 | 0.0 | 0.0 | 569.4 | 227.8 |
| 22 | 174.6 | 27.0 | 162.1 | 0.0 | 0.0 | 0.0 | 428.5 | 171.4 |
| 26 | 154.0 | 23.3 | 131.9 | 0.0 | 0.0 | 0.0 | 362.2 | 144.9 |
| 30 | 90.9 | 23.6 | 81.6 | 0.0 | 0.0 | 0.0 | 230.9 | 92.3 |
| 42 | 6.1 | 14.2 | 43.9 | 0.0 | 0.0 | 0.0 | 83.1 | 33.3 |

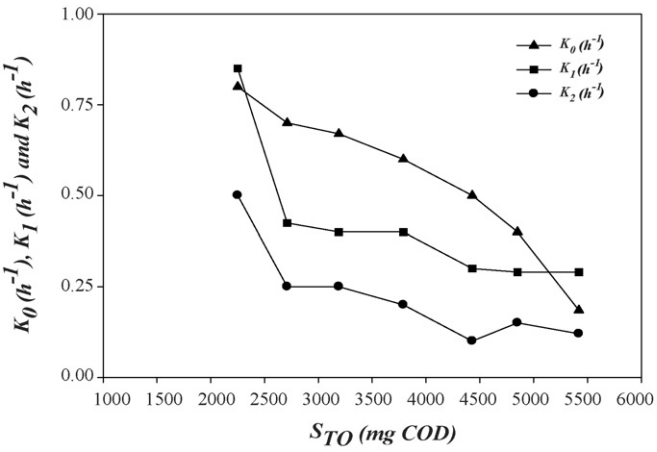


Fig. 8. Variation of the kinetic constants, $K_0$, $K_1$ and $K_2$, obtained with the model as a function of the initial substrate concentration, $S_{T0}$.

the experiments, with values in the range of 7.0–7.2 g VSS/L [4], no differential equation was proposed for biomass concentration evolution. In this model, therefore, it is assumed that the possible accumulation of biomass is muffled either by effluent loss or by its adhesion to the surface of the reactor walls, which is difficult to quantify.

The values of the kinetic constants $K_0$, $K_1$ and $K_2$ for each load tested were determined from the experimental results plotted in Figs. 1–7 using the 2006 Mathcad software (version 13). This software uses the Marquardt multiple-response non-linear regression algorithm [24]. The set of differential equations must be integrated, because the data obtained are integral (taking into account the evolution of concentrations with time). This integration has been carried out using a fourth-order Runge–Kutta algorithm coupled to the regression in order to integrate the equations at the same time.

Fig. 8 illustrates the variation of the values of kinetic constant $K_0$, $K_1$ and $K_2$ as a function of the loading added to the reactor. As can be seen, the three kinetic constants decreased markedly with the load added to the reactor, showing the occurrence of an inhibition process. This behaviour was believed to be due to the high levels of long-chain fatty acids and pectin present in the wastewater studied, substances characterized by its inhibitory effect in anaerobic digestion processes [10,12,18,20,25]. In addition, the kinetic constant $K_2$ showed the lowest values for all the loads studied, which revealed that the methanogenic step was the slowest step in comparison with the other stages of the overall anaerobic process. In any case, the values of the kinetic constants $K_0$ and $K_2$ obtained in the present study were much higher than those reported in the hydrolytic and methanogenic steps of anaerobic digestion of livestock manure and two-phase olive pomace [9,25] for which unstructured segregated first and second-order kinetic models and first-order and Michaelis–Menten equation types, respectively, were considered for the hydrolytic and methanogenic steps, respectively, using various sets of differen-

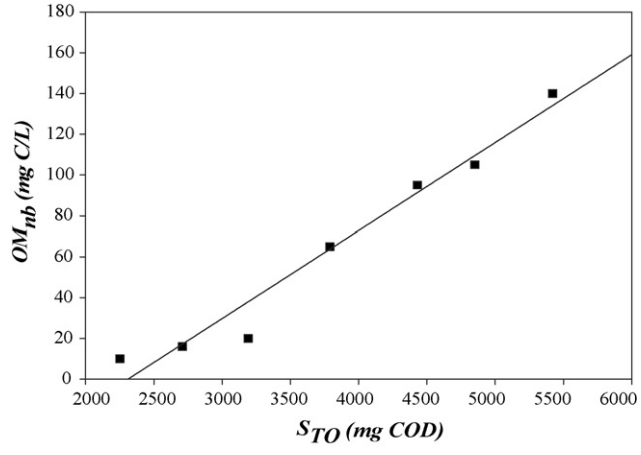


Fig. 9. Variation of the fraction of OM non-biodegradable ($OM_{nb}$) predicted by the model with the initial substrate concentration, $S_{T0}$.

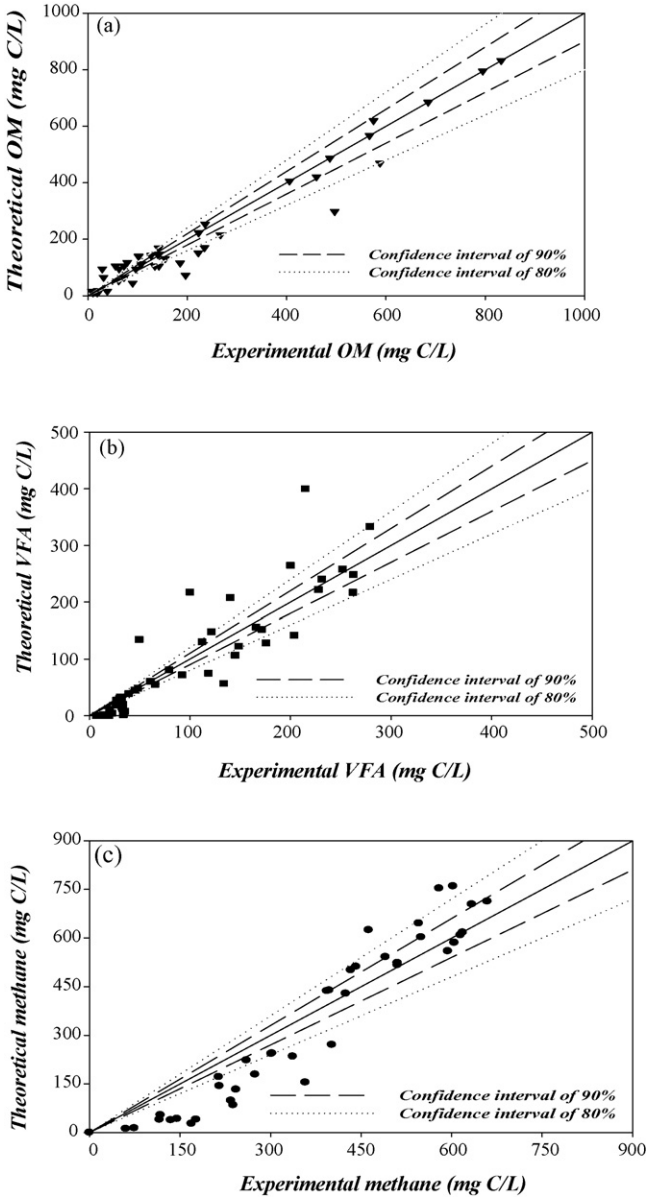


Fig. 10. Comparison between the experimental organic matter (OM) (a), VFA (b) and methane (c) values and the theoretical values as predicted by the model.

tial equations. Moreover, the $K_0$ values obtained in the present work (0.85–0.18 $h^{-1}$) were always higher than those obtained in the hydrolytic stage of batch kinetic experiments codigesting piggery, olive-mill and dairy wastewaters (0.24–0.68 $day^{-1}$) for which microbial growth kinetics of each culture were calculated and used for comparison [26].

Fig. 9 shows the variation of the non-biodegradable organic matter concentration ($OM_{nb}$) values (obtained through the model) with the initial substrate concentration. As can be seen, the non-biodegradable organic matter concentration increased linearly with higher loads, as was expected, with extreme values of 10 and 140 mg C/L for the loads of 2.0 and 5.0 g COD, respectively.

### 3.3. Validation of the kinetic model

Figs. 1–7 compare the evolution of organic matter concentration, VFA and methane (all expressed as C) with the digestion time by plotting the experimental points (◯) and the theoretical curves (solid lines) obtained with the model. The small deviations obtained in all cases (lower than 20% in most cases) demonstrate the suitability of the mathematical model proposed and strongly suggest that this model very accurately describes the variation of organic matter concentration, VFA and methane with time in the anaerobic digestion process studied. Figs. 1–7 also show the variation of the theoretical intermediate product concentrations ($I$) with digestion time, although these products were not experimentally determined because there were so many with different characteristics.

Finally, Fig. 10 compares the experimental values of organic matter concentration, VFA and methane production with the corresponding theoretical values obtained with the model. These calculations were performed so as to give error bands of 10% and 20%. Specifically, the percentages of reproducibility of the model with an error lower than 20% were: 55% for organic matter concentration, 45% for VFA and 60% for methane. Therefore, the deviations obtained between the experimental and theoretical values were lower than 20% in most cases, suggesting that the proposed model can be used to predict the behaviour of this reactor accurately and that the kinetic parameters obtained represent the activity of the microorganisms effecting the anaerobic digestion of this wastewater.

## 4. Conclusions

The results obtained in this study allow the following conclusions to be drawn:

- Anaerobic digestion of wastewater derived from the pressing of orange rind produced during the orange juice production can be described as a simplified three-step reaction scheme: (1) the complex or high-molecular-weight compounds contained in the substrate are hydrolysed and converted into intermediate products of lower molecular weight; (2) these intermediate products are converted to volatile fatty acids; (3) the VFA are transformed into methane by methanogenic microorganisms.
- A mathematical model based on four segregated differential equations was formulated to describe the batch anaerobic digestion of this wastewater, assuming that a fraction of this substrate is non-biodegradable and the afore-mentioned steps follow a first-order kinetics.
- The kinetic constants corresponding to these three stages ($K_0$, $K_1$ and $K_2$) decreased markedly with the load added to the reactor, showing the occurrence of an inhibition process, the decrease being more pronounced for $K_0$, which indicates that the hydrolytic step was the most inhibited.
- The proposed model was validated by comparing the theoretical curves obtained with the corresponding experimental data of organic matter, VFA and methane. The deviations obtained in most cases (less than 20%) demonstrate the suitability of the mathematical model proposed and suggest that this model accurately describes the evolution of organic matter, VFA and methane with time in the anaerobic digestion process of this wastewater.

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