Kinetics for Substrate Utilization and Methane Production during the Mesophilic Anaerobic Digestion of Two Phases Olive Pomace (TPOP)

RAFAEL BORJA,*,[†] ANTONIO MARTÍN,[‡] BÁRBARA RINCÓN,[†] AND FRANCISCO RAPOSO[†]

Instituto de la Grasa (C.S.I.C.), Avda. Padre García Tejero 4, 41012-Sevilla, Spain, and Departamento de Ingeniería Química, Facultad de Ciencias, Campus Universitario de Rabanales, Edificio C-3, Ctra. Madrid-Cádiz, Km 396, 14071 Córdoba, Spain

A kinetic study of the anaerobic digestion process of two phases olive pomace (TPOP) was carried out in a laboratory-scale completely stirred tank reactor at mesophilic temperature (35 °C). The reactor was operated at influent substrate concentrations of 34.5 (substrate I), 81.1 (substrate II), 113.1 (substrate III), and 150.3 g COD/L (substrate IV). The hydraulic retention times (HRT) ranged between 8.3 and 40.0 days for the most diluted substrate (I) and between 10 and 50 days for the other three influent substrate concentrations used (substrates II−IV). The results obtained demonstrated that the rates of substrate uptake and methane production were correlated with the concentration of biodegradable total chemical oxygen demand (COD), through equations of the Michaelis−Menten type. A mass (COD) balance around the reactor allowed the methane yield coefficient and cell maintenance coefficient to be obtained, which gave values of 0.25 L CH_4/g COD_t and 0.25 days^{-1}, respectively. The first one was coincident to that obtained through experimental data of methane production and substrate consumption. The kinetic equations obtained and the proposed mass balance were used to simulate the anaerobic digestion process of TPOP and to obtain the theoretical COD of the reactor and methane production rates. The small deviations obtained (equal or lower than 10%) between the values calculated through the model and experimental ones suggest that the proposed model predicts the behavior of the reactor very accurately.

KEYWORDS: Kinetics; anaerobic digestion; methane production; substrate consumption; two phases olive pomace (TPOP); simulation

INTRODUCTION

A number of kinetic models have been proposed for the process of anaerobic digestion. Early models were based on a single culture system and used the Monod equation or variations (*1−3*). More recently, several dynamic simulation models have been developed based on a continuous multiculture system; these correspond to the major bioconversion steps in anaerobic digestion but again make the assumption that culture growth obeys Monod type kinetics (*4−7*). Doubt has been expressed by several investigators on the validity of applying the Monod equation to waste treatment (*8−10*), as the specific growth rate is expressed only as a function of the concentration of the limiting substrate in the reactor. The equation (*11*) contains no term relating to input substrate concentration; this implies that the effluent substrate concentration is independent of the input concentration. Experimental results do not always agree with

this implication, e.g., the anaerobic digestion of dairy manure (*2*), beef cattle manure at mesophilic and thermophilic temperatures (*12, 13*), rice straw (*14*), or poultry litter (*15*).

Deviation from the Monod relationship in many digestion systems may be due to their complexity. This complexity has necessitated the use of generalized measures of feed and effluent strength, namely, total chemical oxygen demand (COD) and volatile solids (VS), which may not truly reflect the nature of the growth-limiting substrate. Utilizable carbon in the digester is derived from the hydrolysis of polymeric compounds, constituting the waste, by exoenzymes in the extracellular medium or on the surface/vicinity of the microorganisms: only these hydrolyzed, assimilable compounds can be considered as the growth-limiting substrate in terms of the Monod relationship. Extracellular hydrolysis is often considered the rate-limiting step in anaerobic digestion of organic wastes (*7, 16−18*), and for a model to be truly valid, this must be taken into account.

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

* To whom correspondence should be addressed. Tel: 34-95-4 68 96 54. Fax: 34-95-4 69 12 62. E-mail: rborja@cica.es.
† Instituto de la Grasa (C.S.I.C.).
‡ Departamento de Ingeniería Química, Facultad de Ciencias.

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models based on this premise necessarily involve a number of kinetic equations and coefficients making them highly complex, as shown by the reported models (*4–7*). Complexity does not necessarily equate to accuracy, and there is still a strong case in favor of a simpler kinetic treatment based on a single culture system. Methanogenesis is particularly suited to this approach as there is a strong holistic characteristic 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.

On the other hand, the new two phases olive oil mills produce a new and complex aqueous solid residue from the primary centrifugation step that contains the olive vegetation water, commonly called two phases olive mill solid waste (OMSW) or two phases olive pomace (TPOP). This new technology is currently used in 90% of the Spanish olive oil factories as a consequence of the great reduction in the water consumption of the mill. Therefore, the TPOP constitutes the new waste and the majority of waste of these food industries.

The average composition of the TPOP is water (60–70%), lignine (13–15%), cellulose and hemicellulose (18–20%), olive oil retained in the pulp (2.5–3%), and mineral solids (MS) (2.5%). Among their organic components, the major ingredients are as follows: sugars (3%), volatile fatty acids (VFA) (C2–C7) (1%), polyalcohols (0.2%), proteins (1.5%), polyphenols (0.2%), and other pigments (0.5%).

As can be seen, the TPOP has a high organic matter concentration giving an elevated polluting load. The high polluting power and large volumes of solid waste generated (around 2 million tons per year in Spain) can pose large-scale environmental problems, taking into account the 2000 Spanish olive oil factories, most of them located in the Andalusia community (*19*).

A previous paper (*19*) showed the anaerobic digestibility of this solid waste using a laboratory-scale completely stirred tank reactor at mesophilic temperature (35 °C). The reactor operated at influent substrate concentrations of 34.5 (substrate I), 81.1 (substrate II), 113.1 (substrate III), and 150.3 g COD/L (substrate IV) and at hydraulic retention times (HRT) of between 8.3 and 40.0 days for the first feed used (substrate I) and between 10 and 50 days for the other three influent substrate concentrations (substrates II–IV). COD and VS removal efficiencies of 88.4 and 90.9%, respectively, were achieved at an organic loading rate (OLR) of 12.02 g COD/L day for the most concentrated substrate used (substrate IV). The maximum methane production rate was 2.12 L CH$_4$/L day for the above-mentioned OLR and a HRT of 12.5 days. The methane yield coefficients obtained were 0.30, 0.27, 0.23, and 0.20 L methane STP/g COD removed for the substrates I–IV, respectively.

The aim of this work was to carry out a kinetic evaluation of the mesophilic anaerobic digestion of TPOP using a completely stirred tank reactor and four influent substrate concentrations of 34.5 (substrate I), 81.1 (substrate II), 113.1 (substrate III), and 150.3 (substrate IV). All of the data obtained were evaluated and processed altogether. Two kinetic equations for substrate utilization and methane production have been derived from the experimental data. In addition, the nonbiodegradable substrate concentration was also estimated from these data by using the kinetic equations obtained. A mass (COD) balance around the reactor allowed the methane yield coefficient value to be obtained. The proposed models allowed us to predict the behavior of the reactor very accurately.

Table 1. Composition and Features of the Four Concentrations of TPOP Used[a]

	I	II	III	IV
pH	5.6	4.8	5.1	5.8
total COD	34.5	81.1	113.1	150.3
soluble COD	14.5	37.5	49.8	66.5
TVFA	0.70	1.53	2.20	2.90
alkalinity	0.735	1.220	0.960	2.20
TS	40.2	84.8	124.0	165.3
MS	5.6	9.9	15.8	21.1
VS	34.6	74.9	108.2	144.2
TSS	35.2	71.8	106.6	142.2
MSS	4.1	7.5	11.8	15.7
VSS	31.1	64.3	94.8	126.5
total phenolic compds (caffeic acid)	0.61	1.22	1.83	2.44

[a] TVFA (as acetic acid); alkalinity (as CaCO$_3$). All amounts, except pH, are expressed in g/L. Values are averages of five determinations; there was virtually no variation (less than 3%) between analyses.

MATERIALS AND METHODS

Equipment. An anaerobic reactor with a working volume of 1 L equipped with a magnetic stirrer and placed in a thermostatic chamber at 35 °C was used. The reactor was fed daily by means of an external feeder, and liquid effluent was removed daily through a hydraulic seal, comprising 25 cm liquid column, designed to prevent air from entering the reactor and biogas from leaving. This reactor has been described in detail elsewhere (*20*).

The methane volume produced in the process was measured using a 5 L Mariotte reservoir fitted to the reactor. A tightly closed bubbler containing a NaOH solution (3 M) to collect the CO$_2$ produced in the process was intercalated between the two elements. The methane produced displaced a given volume of water from the reservoir, allowing ready determination of the gas (*20*).

Inoculum. The reactor was inoculated with methanogenically active biomass from a laboratory-scale anaerobic reactor processing olive mill wastewater. Its content in total suspended solids (TSS) and volatile suspended solids (VSS) was 59.9 and 41.1 g/L, respectively. A detailed description of the composition and features of the inoculum used is given in a previous paper (*19*).

TPOP. The TPOP used was collected from the Experimental Olive Oil Factory located in the "Instituto de la Grasa" (CSIC) of Sevilla, Spain. Four influent substrate concentrations (substrates I–IV) were prepared by dilution of the most concentrated one. Substrates I–III corresponded to dilutions of 22.9, 53.9, and 75.2% of the most concentrated substrate (IV), respectively. The features and composition of these four TPOPs are summarized in **Table 1**.

Experimental Procedure. The anaerobic reactor was initially charged with 300 mL of distilled water, 500 mL of the inoculum, and 200 mL of a nutrient–trace element solution. The composition of this nutrient–trace element solution is given in detail elsewhere (*21*).

The start-up of the reactor involved stepped increases in COD loading using an influent substrate concentration of 17.2 g COD/L. During this step, the OLR was gradually increased from 0.25 to 1.25 g COD/L day in a 60 day period. A detailed description of the different steps followed during this period is given elsewhere (*19*).

This preliminary step was followed by a series of continuous experiments using feed flow rates of 0.025, 0.035, 0.04, 0.05, 0.06, 0.08, 0.10, and 0.12 L/day of substrate I described in **Table 2**, which correspond to HRTs of 40.0, 28.6, 25.0, 20.0, 16.6, 12.5, 10.0, and 8.3 days, respectively. For the other three substrates used (II–IV), feed flow rates of 0.02, 0.04, 0.06, 0.08, and 0.10 L/day were used, which correspond to HRTs of 50.0, 25.0, 16.6, 12.5, and 10.0 days, respectively. Experiments were carried out using progressive influent substrate concentrations; those corresponding to the most diluted substrate (I) were performed first, and those corresponding to the most concentrated substrate (IV) were performed at the end of the study. In addition, the organic loadings applied in this work were increased in a

Table 2. Most Relevant Steady State Results under Different Experimental Conditions for the Substrate I (COD = 34.5 g/L)[a]

OLR (g COD/L day)	0.86	1.21	1.38	1.72	2.08	2.76	3.45	4.14
HRT (days)	40.0	28.6	25.0	20.0	16.6	12.5	10.0	8.3
r_{CH_4} (L CH₄/L day)	0.240	0.340	0.385	0.470	0.560	0.730	0.910	0.850
COD (g/L)	2.30	2.50	2.74	3.40	3.85	4.20	4.50	5.80
soluble COD (g/L)	0.72	1.20	1.40	1.65	1.90	2.15	2.35	3.80
COD removal (%)	93.3	92.8	92.1	90.1	88.8	87.8	87.0	83.2

[a] r_{CH_4}, volumetric methane production rate (L CH₄/L day); COD, total COD (g/L). Values are the averages of five determinations taken over 5 days after the steady state conditions had been reached. The differences between the observed values were less than 3% in all cases.

Table 3. Steady State Results under Different Experimental Conditions for the Substrate II (COD = 81.1 g/L)[a]

OLR (g COD/L day)	1.62	3.24	4.89	6.49	8.11
HRT (days)	50.0	25.0	16.6	12.5	10.0
r_{CH_4} (L CH₄/L day)	0.430	0.845	1.230	1.545	1.375
COD (g/L)	4.25	4.95	6.85	9.30	11.2
soluble COD (g/L)	2.45	3.15	4.50	7.50	8.80
COD removal (%)	94.8	93.9	91.6	88.5	86.2

[a] r_{CH_4}, volumetric methane production rate (L CH₄/L day); COD, total COD (g/L). Values are the averages of five determinations taken over 5 days after the steady state conditions had been reached. The differences between the observed values were less than 3% in all cases.

Table 4. Most Relevant Steady State Results under Different Experimental Conditions for the Substrate III (COD = 113.1 g/L)[a]

OLR (g COD/L day)	2.26	4.52	6.81	9.05	11.31
HRT (days)	50.0	25.0	16.6	12.5	10.0
r_{CH_4} (L CH₄/L day)	0.560	0.995	1.470	1.870	1.750
COD (g/L)	4.25	6.50	9.65	11.50	14.80
soluble COD (g/L)	2.50	4.00	6.80	8.05	9.75
COD removal (%)	96.2	94.3	91.5	89.8	86.9

[a] r_{CH_4}, volumetric methane production rate (L CH₄/L day); COD, total COD (g/L). Values are the averages of five determinations taken over 5 days after the steady state conditions had been reached. The differences between the observed values were less than 3% in all cases.

stepwise fashion in order to minimize the transient impact on the reactor that might be induced by a sudden increase in loadings.

Once steady state conditions were achieved at each feed flow rate, the daily volume of methane produced, total and soluble COD, pH, total VFAs (TVFA), and VS of the different effluents were determined. The samples were collected and analyzed for at least 5 consecutive days. The steady state value of a given parameter was taken as the average of these consecutive measurements for that parameter when the deviations between the observed values were less than 3% in all cases. Each experiment had a duration of 2–3 times the corresponding HRT.

Chemical Analyses. The parameters were determined as follows: total and soluble COD, pH, total solids (TS), MS, VS, TSS, mineral suspended solids (MSS), VSS, TVFA, alkalinity, and total phenolic compounds. All analyses were carried out according to the recommendations of the Standard Methods of APHA (*22*).

In each steady state experiment, samples were collected and the above parameters were analyzed. The pH and gas volume were determined daily, while the remaining parameters were measured at least five times per week on five different samples taken on different days to ensure that representative data were obtained.

RESULTS AND DISCUSSION

Tables 2–5 summarize the most relevant steady state operating results including HRT, OLR, methane production rates (r_{CH_4}), and total and soluble CODs for substrates I–IV,

Table 5. Most Relevant Steady State Results under Different Experimental Conditions for the Substrate IV (COD = 150.3 g/L)[a]

OLR (g COD/L day)	3.00	6.01	9.05	12.02	15.03
HRT (days)	50.0	25.0	16.6	12.5	10.0
r_{CH_4} (L CH₄/L day)	0.590	1.130	1.640	2.120	2.050
COD (g/L)	4.80	9.05	12.95	17.50	25.70
soluble COD (g/L)	3.05	6.00	8.25	11.30	15.05
COD removal (%)	96.8	94.0	91.4	88.4	82.9

[a] r_{CH_4}, volumetric methane production rate (L CH₄/L day); COD, total COD (g/L). Values are the averages of five determinations taken over 5 days after the steady state conditions had been reached. The differences between the observed values were less than 3% in all cases.

respectively. Other experimental data (pH, TVFA, alkalinity, and VS) obtained in the anaerobic digestion process of these four influent substrate concentrations are described in detail in a previous paper (*19*).

As can be obtained from **Tables 2−5**, the percentage of COD removal decreased with increased OLR for the four influent substrate concentrations studied. The percentage of COD removal decreased from 93.3 to 83.2% when OLR increased from 0.86 to 4.14 g COD/L day for the most diluted influent used (substrate I). In the same way, a COD removal value of 88.4% was obtained at an OLR of 12.02 g COD/L d, when the most concentrated influent (substrate IV) was processed (*19*).

Moreover, the above-mentioned work (*19*) clearly demonstrated the progressive adaptation of the biomass to an increase in substrate concentration, as well as a gradual increase in the methanogenic activity of the anaerobic sludge with the advance of the experiments. Therefore, all of these results also indicated that anaerobic digestion is a practical and promising alternative for the treatment of TPOP. Besides this, it was observed that an average of 83% of the organic matter added to the reactor is degraded during the anaerobic digestion of TPOP at mesophilic temperature.

The experimental methane yield coefficients were calculated from the values of methane production rates and COD removals. Values of 0.30, 0.27, 0.23, and 0.20 L CH₄/g COD removed were obtained for the influent substrate concentrations I−IV, respectively (*19*). All of these values agreed with the data reported in the literature for anaerobic treatments of food industry wastewaters (*23−26*).

On the other hand, taking into account the experimental setup (completely stirred tank reactor) and the procedure used in the experiments, the hypothesis of complete mixture for both the liquid and the solid phases can be established. As, in addition, the steady state conditions were achieved for each experiment carried out, the volumetric rate of substrate uptake (total COD) or substrate removal rate can be obtained from the equation:

$$-(r_{COD}) = (COD_0 - COD)/(HRT) \qquad (1)$$

where COD_0 is the incoming total COD concentration; COD is the outgoing total COD concentration or total COD concentration in the reactor; and HRT is the HRT. The minus sign in r_{COD} only has physical meaning, and it indicates that COD concentration diminishes when increasing the HRT.

Equation 1 allows the direct calculation of the substrate utilization rate in the reactor based on experimental data observed. This equation was used by Shieh et al. (*27*) to describe the anaerobic digestion of synthetic wastewater containing glucose as the sole carbon source. This equation was also used for obtaining the substrate utilization rates in the anaerobic digestion process of brewery (*28*), fruit processing (*29*),
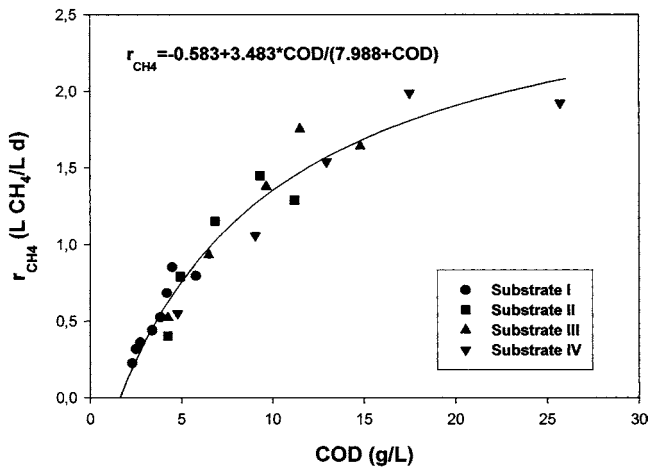
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Figure 1. Variation of the methane production rate, r_{CH_4} (L CH$_4$/L day), as a function of the total COD concentration in the reactor (g/L) for the four influent substrate concentrations used.
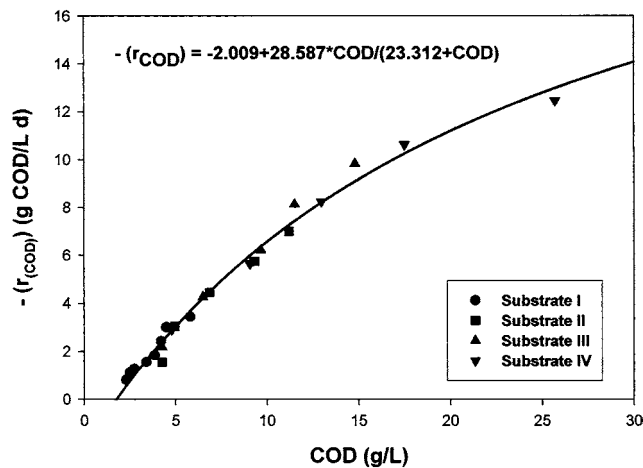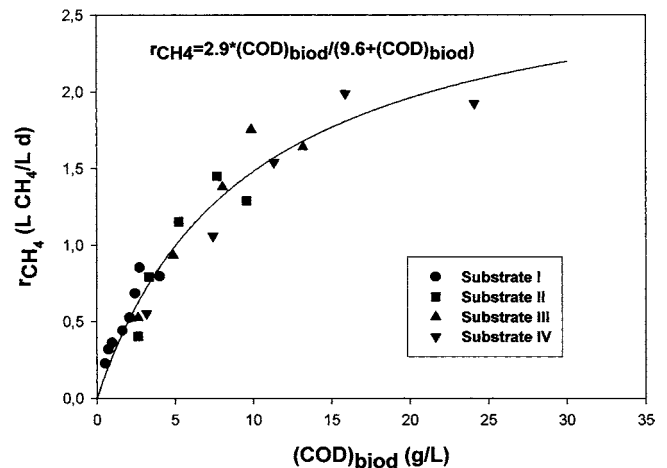

Figure 3. Variation of the methane production rate, r_{CH_4} (L CH$_4$/L day), as a function of the biodegradable total COD concentration in the reactor (g/L) for all the experiments carried out.

Figure 2. Variation of the substrate removal rate, r_{COD} (g COD/L day), as a function of the total COD concentration in the reactor (g/L) for the four influent substrate concentrations used.
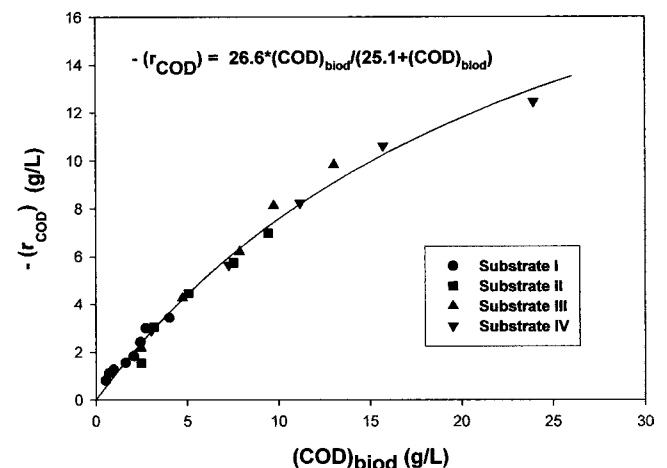

Figure 4. Variation of the substrate removal rate, r_{COD} (g COD/L day), as a function of the biodegradable total COD concentration in the reactor (g/L) for all the experiments carried out.

slaughterhouse (*30*), and olive mill wastewaters (*31*) by using completely mixed reactors with biomass immobilized on different clays as bacterial supports.

Analogously, the volumetric methane production rate (r_{CH_4}) can be obtained through the expression:

$$r_{CH_4} = V_{CH_4}/V_R \quad (2)$$

where V_{CH_4} is the daily methane production (L CH$_4$/day) and V_R is the reactor volume (L).

A plot of both the volumetric methane production rates, r_{CH_4}, and the volumetric substrate utilization rates vs the total COD give hyperbolic curves whose intercepts on the *x*-axis are not equal to zero, as illustrated in **Figures 1** and **2**. This fact clearly shows that a fraction of substrate is not biodegradable even working at HRTs as high as 40 days.

The concentration of nonbiodegradable substrate was estimated graphically from **Figures 1** and **2** on the basis that this value is coincident with the total COD concentration that makes zero the methane production and substrate utilization rates (*32*). Therefore, the experimental values of total COD were corrected by subtracting the fraction of nonbiodegradable substrate (1.69 g COD/L) in order to obtain the biodegradable COD values (COD$_{biod}$).

The observed methane production rates and substrate utilization rates plotted as a function of the biodegradable COD concentration are illustrated in **Figures 3** and **4**. It can be seen from these figures that both methane production and substrate utilization rates fit the Michaelis–Menten kinetic model, which is a hyperbolic function, quite well. By using the version 2001 SigmaPlot software, the following two equations were obtained

$$r_{CH_4} = 2.9(COD_{biod})/(9.6 + (COD_{biod})) \quad (3)$$

$$-(r_{COD}) = 26.6(COD_{biod})/(25.1 + (COD_{biod})) \quad (4)$$

To make a COD balance around the reactor, a hypothesis can be established as follows: the anaerobic digester operates at steady state conditions because the total and soluble CODs in the reactor were maintained virtually constant for all of the HRTs assayed and initial substrate concentrations studied.

By making a COD balance around the reactor, the following equation is obtained

$$q(COD_0 - COD) = a\, r_{CH_4}V_R + b\, q(COD - COD_s) +$$
$$c(COD - COD_s)V_R \quad (5)$$

where q is the flow rate (L/day); COD_s is the outgoing soluble COD or soluble COD in the reactor; and a, b, and c are three

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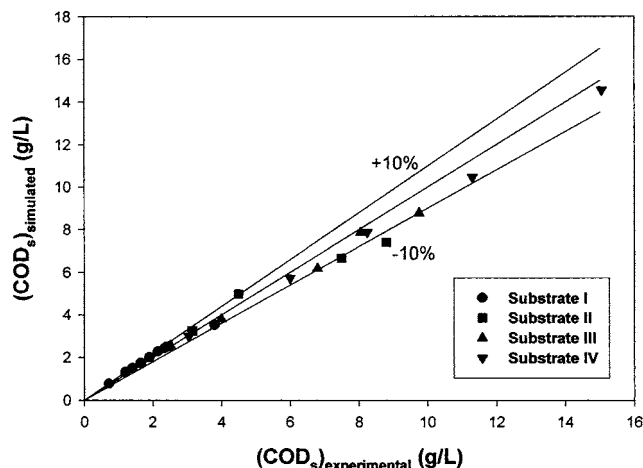
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Figure 5. Comparison between the values of experimental soluble COD concentration (g/L) in the reactor and the theoretical values obtained by simulation of the four influent substrate concentrations used.
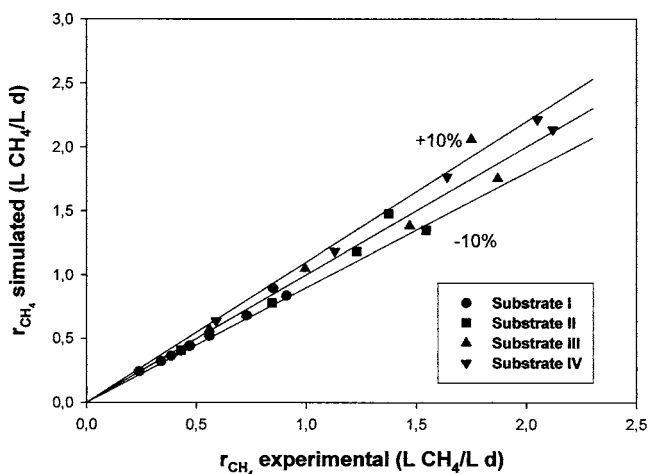

Figure 6. Comparison between the experimental methane production rates, r_{CH_4} (L CH$_4$/L day) and the theoretical rates obtained by simulation of the four influent substrate concentrations used.

parameters, from which a and c have the dimensions of g COD/L CH$_4$ and days^{-1}, respectively.

The first member of eq 5 represents the global consumption of COD. The second member represents the different fractions in which the removed COD is transformed. These are methane formation, biomass generation, and cell maintenance, respectively.

Given the experimental difficulty to measure the viable biomass, because this is mixed with the nondigested solids remaining in the reactor, it is assumed that the outgoing biomass concentration or biomass concentration in the reactor is proportional to the difference between the outgoing total and soluble COD (COD − COD$_s$). In the same way, the third term of the second member of eq 5 represents the consumption of COD necessary for cell maintenance.

From the experimental data (**Tables 2−5**) and using a multiple linear regression, the values for the parameters a, b, and c were obtained as follows: $a = 3.93$ g COD/L CH$_4$; $b = 0.12$; and $c = 0.25$ days^{-1}.

The parameter a (3.93 g COD/L CH$_4$) is equivalent to the inverse of the methane yield coefficient (Y_p). Thus, the estimated methane yield coefficient obtained was found to be 0.25 L CH$_4$/g COD. This value is totally coincident with that obtained from the experimental data (**Tables 2−5**) by fitting all of the (daily

methane production, grams of COD removed) value pairs to a straight line. Taking into account that theoretically 0.35 L of methane is produced per gram of COD removed, when assuming that all of the incoming COD is transformed into methane and considering null the biomass growth and cell maintenance (*33*), the effectiveness of the anaerobic reactor in converting TPOP into methane (71.4% of theoretical value) at mesophilic temperature is also clearly demonstrated.

On the other hand, the parameter b was lower than 1 (0.12) and c was relatively low (0.25), which indicates a low energetic yield for the process. In any case, the above-mentioned equation should be considered a semiempiric equation that fit well the experimental data.

Finally, eqs 3−5 were used to simulate the experimental behavior of the reactor by using the 2001 version Mathcad Proffesional Software (*34*). In this way, the simulated or theoretical values of soluble COD (COD$_s$) and methane production rates (r_{CH_4}) were obtained and compared with those experimental ones (**Tables 2−5**). **Figures 5** and **6** show plots of the simulated and experimental values of the outgoing soluble COD and methane production rates, respectively. The small deviations obtained (lower than 10%) in both cases suggest that the proposed models and COD balance predict the behavior of this reactor for the treatment of TPOP very accurately and that the parameters obtained represent the activity of the microorganisms affecting the anaerobic digestion of this waste at mesophilic temperature.

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