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Kinetic Analysis of the Psychrophilic Anaerobic Digestion of Wastewater Derived from the Production of Proteins from Extracted Sunflower Flour

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A kinetic analysis of the anaerobic digestion process of wastewater derived from the production of protein isolates from extracted sunflower flour was carried out. The digestion was conducted in a laboratory-scale fluidized bed reactor with saponite (magnesium silicate) as support for the mediating bacteria at psychrophilic temperature (15−19 °C). Soluble chemical oxygen demand (COD_s) removal efficiencies in the range of 95.9−69.0% were achieved in the reactor at organic loading rates (OLR) of between 0.57 and 2.49 g total COD (COD_t)/L d, hydraulic retention times (HRT) of between 20.0 and 4.5 days, and average feed total COD concentration of 11.3 g/L. The yield coefficient of methane production was 0.32 L of methane (at STP) per gram of COD_t removed. The total volatile fatty acid (TVFA) levels and the TVFA/alkalinity ratio were lower than the suggested limits for digester failure for OLR and HRT up to 2.26 g COD_t/L d and 5.0 days, respectively. The specific rate of substrate uptake, r (g COD_s/g VSS d), correlated with the concentration of biodegradable substrate, S (g COD_s/L), through an equation of the Michaelis−Menten type. The maximum substrate utilization rate, k, and the Michaelis constant, K_s, were found to be 0.125 g COD_s/g VSS d and 124 mg COD_s/L, respectively. This proposed model predicted the behavior of the reactor very accurately showing deviations lower than 10% between the experimental and theoretical values of substrate uptake rates. A mass (COD_t) balance around the reactor allowed the COD equivalent of methane volume (W_{CH_4}) to be obtained, which gave a value of 2.89 g COD_t/L CH_4, which was virtually coincident with the theoretical value of 2.86 g COD_t/L CH_4.

KEYWORDS: Kinetic analysis; anaerobic digestion; psychrophilic temperature; wastewater; protein isolates; extracted sunflower flour

INTRODUCTION

Anaerobic digestion of organic waste is an established process for biogas production and organic matter removal, and it has been employed for many years in mesophilic (20−45 °C) and thermophilic (45−60 °C) temperature ranges. The conversion of organic matter into biogas at low temperatures (<20 °C) is referred to as psychrophilic anaerobic digestion (*1*, *2*). Psychrophilic anaerobic digestion has not been studied extensively because it is a slow and difficult process (*3*).

However, under psychrophilic conditions anaerobic treatment of wastewater may give some advantages in energy economy in comparison with mesophilic and thermophilic digestion. So, high-rate anaerobic treatment systems at psychrophilic temperatures have been recently developed (*4*, *5*). The details of the low temperature degradation pathways of diverse substrates and the composition of the microbial communities in various methanogenic environments are still not completely known. A number of investigations of degradation pathways in tundra wetland soil (*6*), forest soils and leaf litter (*7*−*9*), prairie soil (*10*), pond silt (*11*, *12*), lake sediments (*13*, *14*), and pig and cattle manure (*15*, *16*) have shown that acetate is often formed as a result of homoacetogenesis upon addition of H_2/CO_2. Under these conditions, acetoclastic methanogenesis may be the main pathway of methane formation (*5*).

The wastewaters produced in the different steps of the manufacturing process of protein isolates from extracted sunflower flour have organic matter concentrations of between 2 and 45 g COD_t/L, giving a polluting load of the final wastewater of the whole process of between 10 and 15 g COD_t/L (*17*). The high polluting power and large volumes of wastewaters generated (30−40 L/kg of processed flour) can pose large-scale environmental problems, taking into account the 4−5 millions

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of metric tons of sunflower flour produced in Spain, 50% of which is produced in the Andalusia community.

Previous works of anaerobic purification of this wastewater carried out in a laboratory-scale fluidized bed reactor operating at mesophilic temperature (35 °C) showed total chemical oxygen demand (COD) removal efficiencies in the range of 98.3−80.0% at organic loading rates (OLR) of between 0.6 and 9.3 g COD_t/L d and hydraulic retention times (HRT) of between 20.0 and 1.1 days. A methane yield coefficient of 0.33 L methane (at STP) per gram of COD_t removed was obtained in this experiment, and the value was virtually independent of the OLR applied (*17*).

The aim of this work was to carry out a kinetic evaluation of the psychrophilic anaerobic digestion process of wastewater derived from the production of protein isolates from extracted sunflower flour by using a fluidized bed reactor containing microorganisms immobilized on saponite (magnesium silicate). This report discusses a laboratory-scale investigation with emphasis placed on the evaluation of substrate utilization and gas production rates under different operating conditions at psychrophilic temperature (15−19 °C). On the other hand, purifying procedures involving fluidized beds require sturdy supports of low apparent density in order to reduce power consumption, which is the case with the medium assayed here.

MATERIALS AND METHODS

Equipment. The fluidized-bed reactor consisted basically of a cone-shaped glass vessel with a working volume of 1.0 liter. The reactor column itself had a height of 40 cm and an average internal diameter of 6 cm. The reactor had an upper settling zone designed to minimize loss of the biomass or the solids acting as supports for the microorganisms. Effluent was recycled from the settlement zone to the bottom of the reactor at a constant rate of 10 L/h, enough to provide complete fluidization of the biomass. The reactor was fed daily by means of an external feeder, and liquid effluent was removed daily through a hydraulic seal, comprising a 25-cm liquid column, designed to prevent air from entering the reactor and biogas from leaving it. This reactor has been described in great detail elsewhere (*18*).

The methane volume produced in the process was measured using 5-L Mariotte reservoirs 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. The operating temperature of the reactor, 15−19 °C, was maintained as constant by means of an external water jacket through which water from a thermostatic bath circulated.

Support Material. Clay particles of saponite (magnesium silicate) of 0.4−0.8 mm diameter were used as the growth support material. The main characteristics of this packing medium were low fragility, medium porosity (19%), low apparent density (0.55 g/mL), and a high specific surface area (200 m^2/g), which facilitated attachment of anaerobic microorganisms (*19, 20*). It was selected because of its favorable kinetic behavior in previous experiments with other types of food industry wastewaters (*21*−*23*). A detailed description of the composition and features of this support material is given elsewhere (*24*).

Wastewater. The wastewater used was previously homogenized in an equalization tank to minimize any variation that might occur as a result of the batch nature of the manufacturing process. The main features of this wastewater are summarized in **Table 1**, which lists the average values of five separate analyses; there was virtually no variation (less than 5%) between analyses.

Inoculum. The reactor was inoculated with methanogenically active biomass from an industrial anaerobic reactor processing brewery wastewater. Its content in total suspended solids (TSS) and volatile suspended solids (VSS) was 58.9 and 40.2 g/L, respectively. A detailed description of the composition and features of the inoculum used are given in a previous paper (*25*).

Table 1. Composition and Features of the Wastewater[a]

pH	4.8
total chemical oxygen demand (COD_t)	11.3 g/L
soluble chemical oxygen demand (COD_s)	10.9 g/L
total suspended solids (TSS)	1.30 g/L
mineral suspended solids (MSS)	0.53 g/L
volatile suspended solids (VSS)	0.77 g/L
total solids (TS)	8.50 g/L
mineral solids (MS)	2.70 g/L
volatile solids (VS)	5.80 g/L
total volatile fatty acids (TVFA, as acetic acid)	0.86 g/L
alkalinity (as $CaCO_3$)	0.12 g/L

[a] Values are averages of five determinations; there was virtually no variation (less than 5%) between analyses.

Experimental Procedure. The reactor was initially charged with 425 mL of distilled water, 375 mL of the inoculum, 200 mL of a nutrient−trace element solution, and 15 g of the support. Although larger amounts of support provided attachment sites for increased amounts of biomass, they also increased the apparent viscosity of the medium and hence hindered mass transfer and slowed biodegradation. The composition of the nutrient−trace element solution used at the start-up of the reactor can be found elsewhere (*17*).

The start-up of the reactor involved stepped increases in COD loading and substrate concentration. During this period the organic loading rate was gradually increased from 0.1 to 0.3 g COD_t/L d between days 1 and 15, 0.4 g COD_t/L d between days 16 and 30, 0.6 g COD_t/L d between days 31 and 45, and finally 0.7 g COD_t/L d between 46 and 60 days. During these four steps of the acclimatization stage, the influent total COD concentrations were 2.8, 5.6, 8.4, and 11.3 g/L, respectively.

This stepped start-up was followed by a series of continuous experiments using feed flow rates of 50, 75, 100, 125, 150, 175, 200, and 220 mL/d of the wastewater described in **Table 1**, which correspond to hydraulic retention times (HRTs) of 20.0, 13.3, 10.0, 8.0, 6.7, 5.7, 5.0, and 4.5 d, respectively. The bacterial biomass concentration remained virtually constant at 15.0 g VSS/L throughout the experiments. The biomass concentration (g VSS/L) was estimated according to the recommedation of Chen et al. (*26*).

Once steady-state conditions were achieved at each feed flow rate, the daily volume of methane produced, total and soluble COD, pH, total volatile fatty acids, and alkalinity of the different effluents obtained 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× the corresponding HRT.

The organic loadings applied in this work were increased in a stepwise fashion in order to minimize the transient impact on the reactor that might be induced by a sudden increase in loadings.

Chemical Analyses. The following parameters were analyzed according to Standard Methods (*27*): total and soluble COD, pH, total solids (TS), mineral solids (MS), volatile solids (VS), total suspended solids (TSS), mineral suspended solids (MSS), volatile suspended solids (VSS), total volatile fatty acids (TVFA), and alkalinity.

RESULTS AND DISCUSSION

Operational Parameters. Table 2 summarizes the steady-state operating results including HRT, organic loading rates (OLR), total and soluble CODs, TVFA, and alkalinity of the effluents and daily methane productions. As can be seen, for HRT values higher than or equal to 5.0 days, the pH in the reactor remained within the optimal range for methanogenic bacteria with 7.9 and 6.7 as extreme values. For an HRT of 4.5 days, the pH decreased until reaching a value of 6.7. In consequence, an HRT lower than 4.5 days could cause acidification of the reactor, when it is operated at the psychrophilic range of temperature. For HRT values over 5.0 d, TVFA

Table 2. Steady-State Results Obtained under Different Experimental Conditions[a]

HRT (days)	OLR (g COD_t/L d)	COD_{total} (mg/L)	$COD_{soluble}$ (mg/L)	pH	TVFA (mg acetic a./L)	alkalinity (mg $CaCO_3$/L)	$q_{methane}$ (L/d)
20.0	0.57	980	450	7.9	120	2190	0.206
13.3	0.85	1020	480	7.7	140	1530	0.280
10.0	1.13	1240	590	8.0	170	1560	0.366
8.0	1.41	2190	705	7.0	390	1300	0.464
6.7	1.70	2760	1060	6.9	225	1210	0.520
5.7	1.98	3715	1480	6.8	340	1410	0.560
5.0	2.26	5500	2900	6.7	1100	1450	0.425
4.5	2.49	5890	3380	6.6	1240	1330	0.460

[a] Values are averages of 6 determinations taken over 6 days after the steady-state conditions had been reached. The differences between the observed values were less than 4% in all cases. Abbreviations used: HRT, hydraulic retention time; OLR, organic loading rate; TVFA, total volatile fatty acids; $q_{methane}$, daily methane production.

concentration increased very slightly with a decreasing HRT. For HRT values less than 5.0 days, the TVFA concentration increased sharply, achieving a maximum value of 1240 mg/L (as acetic acid) at an HRT of 4.5 days; this increase was concomitant with the decrease in pH. Although a failure was not observed at this HRT, methane production rate was something depressed at the short HRT. In contrast, high gas yields and process stability were always observed in the mesophilic anaerobic treatment of this wastewater at identical HRTs (*17*).

On the other hand, between HRTs of 20.0 and 5.7 days the TVFA/alkalinity ratio was found to be lower than the failure limit (0.3−0.4) value (*28*). However, at a HRT of 4.5 days and OLR of 2.49 g COD_t/L d, a considerable increase of this ratio was observed in the reactor (0.93).

One of the major contributing factors to the failure of the psychrophilic reactor at a 4.5 day HRT is the build-up of longer chain volatile fatty acids. This is indicative of carbon flow through to methane being interrupted by an inhibition of the hydrogen-producing acetogenic bacteria, which are primarily responsible for the breakdown of these compounds to acetic acid. If hydrogen is not being effectively removed from solution by the activity of the autotrophic methanogens then inhibition of this reaction takes place. Failure at the 4.5 day HRT in the reactor might therefore be attributed directly to a failure at some point in this chain of reactions, either directly or by feedback inhibition (*28, 29*).

Biodegradability. As can be observed from the data given in **Table 2**, the reactor was efficient in terms of soluble COD removals. Between HRTs of 20.0 and 5.7 days, soluble COD removal decreased slightly from 95.9 to 86.4%. At an HRT of 4.5 days a marked difference in efficiency was observed (69.0%). These COD removal efficiencies were something lower than those observed at mesophilic temperature (*17*).

Methane Yield Coefficient. The experimental data listed in **Table 2** and the influent substrate concentration were used to determine the methane yield coefficient. By fitting the (daily methane production, g COD_t removed) value pairs to a straight line, the average yield coefficient under standard temperature and pressure (STP) conditions was found to be 0.32 L CH_4 STP/g COD_t removed. This agrees with data reported in the literature (*29*). This value of the methane yield coefficient was only somewhat lower than that obtained at mesophilic temperature (0.33 L CH_4/g COD_t removed). Taking into account that, theoretically, 0.35 L of methane is produced per gram of COD_t removed when the starting compound is glucose (*30*), the effectiveness of the anaerobic reactor in converting wastewater derived from the production of protein isolates from extracted sunflower flour into methane at psychrophilic temperature is also clearly demonstrated.

Kinetic Analysis. The following two hypotheses can be established: the anaerobic reactor operates at steady-state conditions because the VSS concentration in the reactor and the effluent soluble CODs were maintained virtually constant for all the flow rates assayed.

Although the feeding carries suspended solids, the quantity is very small, and it is supposed that all of them are biodegraded in the reactor; this is equal to supposing that the suspended solids content of the effluent corresponds to the biomass generated.

Making a COD balance around the reactor, the following equation is obtained:

$$(COD_t)_0 = (COD_s)_e + (COD)_{biogas} + (COD_{VSS})_e \quad (1)$$

where $(COD_t)_0$ is the incoming total COD concentration; $(COD_s)_e$ is the outgoing soluble COD; $(COD)_{biogas}$ is the fraction of COD converted into biogas; and $(COD_{VSS})_e$ is the fraction of COD converted into biomass.

The above equation can be transformed into the following:

$$q\,S_{t0} = q\,S_{se} + q_{CH_4}\,W_{CH_4} + q\,[S_{te} - S_{se}] \quad (2)$$

where q is the flow rate (L/day); S_{t0} is the feed total COD concentration (g COD_t/L); S_{te} is the effluent total COD concentration (g COD_t/L); S_{se} is the effluent soluble COD concentration (g COD_s/L); q_{CH_4} is the daily methane production (L methane/day); and W_{CH_4} is the methane equivalent of COD (g COD_t/L CH_4).

From eq 2 the following can be obtained:

$$q\,S_{t0} = q_{CH_4}\,W_{CH_4} + q\,S_{te} \quad (3)$$

By grouping terms and dividing by the flow rate, q, the following equation can be obtained:

$$(S_{t0} - S_{te}) = W_{CH_4}\,(q_{CH_4}/q) \quad (4)$$

According to eq 4 a plot of the $(S_{t0} - S_{te})$ versus the quotient (q_{CH_4}/q) should give a straight line of slope equal to W_{CH_4}, whose theoretical value is 2.86 g COD_t/L CH_4, and intercept on the y-axis is equal to zero, as illustrated in **Figure 1**. From **Figure 1**, it is estimated that $W_{CH_4} = 2.89$ g COD_t/L, which was found to be very similar to the theoretical one. As can be seen in **Figure 1**, the points fit a straight line with intercept zero, which strongly suggests the validity of the proposed model.

Each term of the second member of the previous COD balance (eq 1) can be referred to the incoming COD_t, and in this way can be calculated the percentage of the incoming COD_t that is converted into methane and biomass, with the rest being the fraction that goes out with the effluent. **Figure 2** shows the variation of the percentage of COD_t converted into biogas, biomass, and COD that leaves with the effluent (not removed) as a function of the hydraulic retention time (HRT). As can be

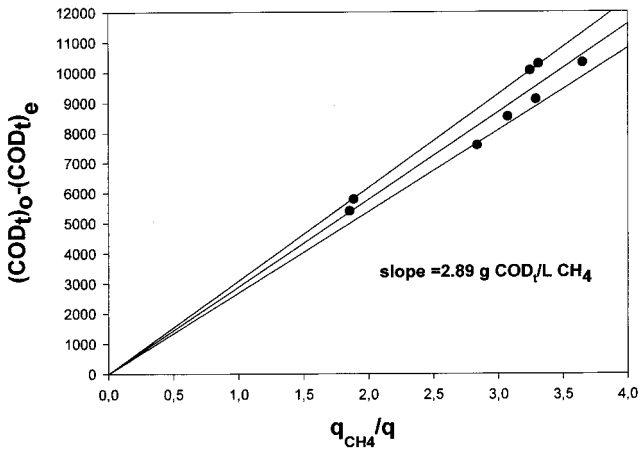

Figure 1. Variation of the differences between the total COD of the influent and effluent ($(COD_t)_0 - (COD_t)_e$) as a function of the quotient between the daily methane production and influent flow-rate (q_{CH_4}/q).

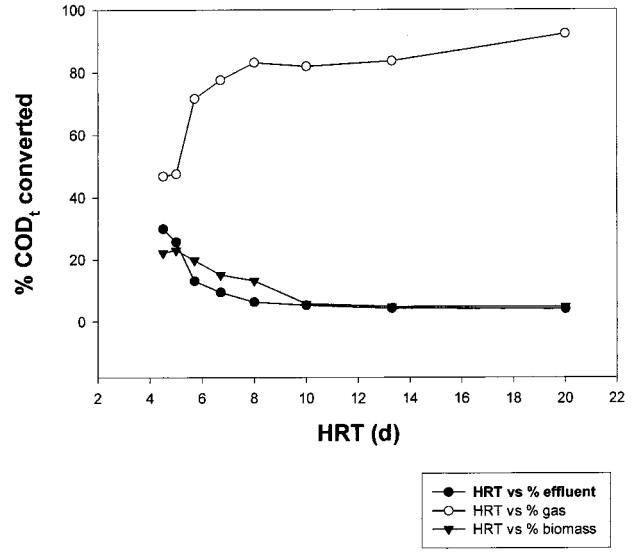

Figure 2. Variation of the percentage of incoming total COD converted into methane, biomass and COD that leaves with the effluent (not removed) as a function of the hydraulic retention time (HRT).

seen for HRTs lower than 5.7 d, the percentage of COD_t that converts into biomass approached 20%, which clearly indicates the growth of hydrolytic and acidogenic bacteria, because the reactor is acidifying and destabilizing. In contrast, for HRT higher than 8 days, the percentage of COD_t transformed into biomass is lower than 10%, which indicates clear methanogenic conditions. According to Stronach et al. (*31*), the maximum yield coefficients of acidogenic and methanogenic bacteria are 0.18 and 0.03 g VSS/g COD_t, respectively. As a mixed culture was used in this study, it is difficult to evaluate the kinetics of these two distinct populations of microorganisms. However, it is obvious that proper control of the methanogenic phase is a key step for successful reactor performance because of the lower substrate yield coefficient of methanogens than that of acidogenic bacteria. Values (Y = 0.15 and 0.16 g VSS/g COD_t) similar to those obtained in this work for HRTs lower than 5.7 days were reported in the literature for anaerobic digestion of whey permeate and ice-cream wastewater using UASB reactors (*31, 32*).

In the above-given equations S denotes the concentration of biodegradable substrate; however, the experimental method used to determine the substrate concentration (total and soluble COD

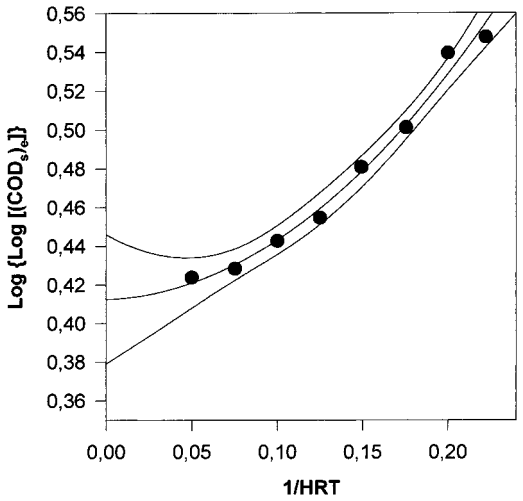

Figure 3. Estimation of the fraction of nonbiodegradable organic matter (soluble COD) contained in the wastewater.

analysis) does not distinguish between biodegradable and nonbiodegradable substrate. The experimental values of soluble COD given in **Table 2** must be corrected by subtracting the fraction of nonbiodegradable substrate. **Figure 3** shows the graphical estimation of the amount of nonbiodegradable substrate on the basis of the relationship between log [log$(COD)_{s\,e}$] and 1/(hydraulic retention time) (*33*). By least-squares fitting of the two variables an intercept of 0.385 g COD_s/L (correlation coefficient = 0.995) was calculated, which corresponds to an infinite HRT; thus, this can be assumed to be the concentration of nonbiodegradable substrate.

On the other hand, according to the Michaelis−Menten kinetic model, the specific substrate utilization rate, r, is related with the biodegradable substrate concentration, S, by the following equation:

$$r = kS/(K_s + S) \tag{5}$$

where k is the maximum substrate utilization rate (g COD_s/g VSS day) and K_s is the Michaelis constant (g COD_s/L). At the steady-state, r can be obtained by the following equation (*18, 33*):

$$r = (S_0 - S)/\theta\, X \tag{6}$$

where θ is the hydraulic retention time (HRT) ($\theta = V/q$, V being the reactor volume in liters) and X is the biomass concentration in the reactor (g VSS/L).

Therefore, by combining eqs 5 and 6, it is possible to determine experimentally whether the Michaelis−Menten expression is applicable for the description of substrate utilization in the anaerobic fluidized-bed reactor

$$r = (S_0 - S)/\theta\, X = k\, S/(K_s + S) \tag{7}$$

The observed substrate utilization rates plotted according to eq 7 as a function of steady-state biodegradable effluent COD concentration, S, are illustrated in **Figure 4**. It can be seen from this figure that the substrate utilization rates fit the Michaelis−Menten expression, which is a hyperbolic function, quite well.

Both k and K_s can be determined by plotting S/r as a function of S

$$S/r = (K_s/k) + (1/k)\, S \tag{8}$$

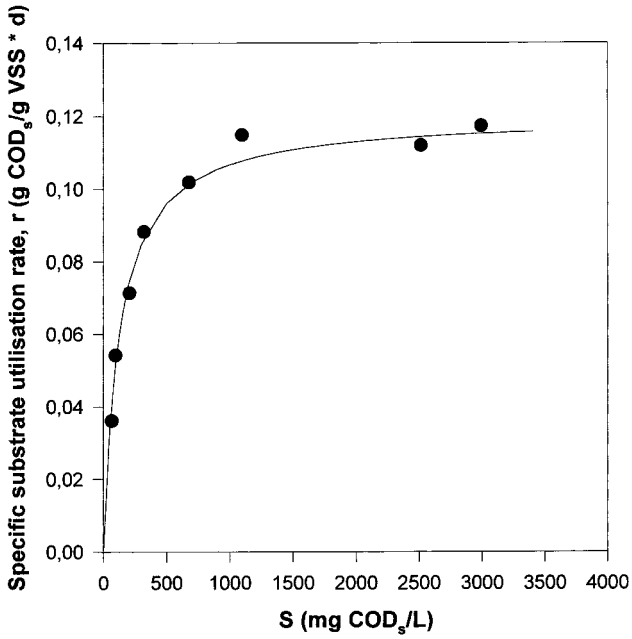

Figure 4. Variation of the specific substrate utilization rate, r (g COD_s/g VSS d), as a function of the concentration of biodegradable substrate, S (mg COD_s/L).

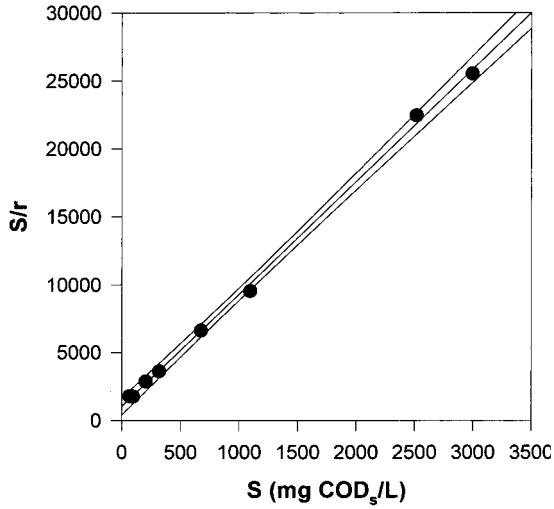

Figure 5. The plot of the quotient S/r versus the concentration of biodegradable substrate, S, for determination of the maximum substrate utilization rate (k) and Michaelis constant (K_s).

From this linearized equation, k can be calculated from the slope of the straight line and K_s can be calculated from the intercept on the y-axis, as illustrated in **Figure 5**. From this figure, it is estimated that $k = 0.125$ g COD_s/g VSS day and $K_s = 124$ mg COD_s/L. Substitution of these values into eq 5 allowed the theoretical rate of substrate uptake to be determined. **Figure 6** shows a plot of the theoretical and experimental values of the specific substrate removal rates. The small deviations obtained (lower than 10%) suggest that the proposed model predicts the behavior of this reactor for this wastewater very accurately and that the kinetic parameters obtained represent the activity of the microorganisms effecting the anaerobic digestion of this wastewater at psychrophilic temperatures.

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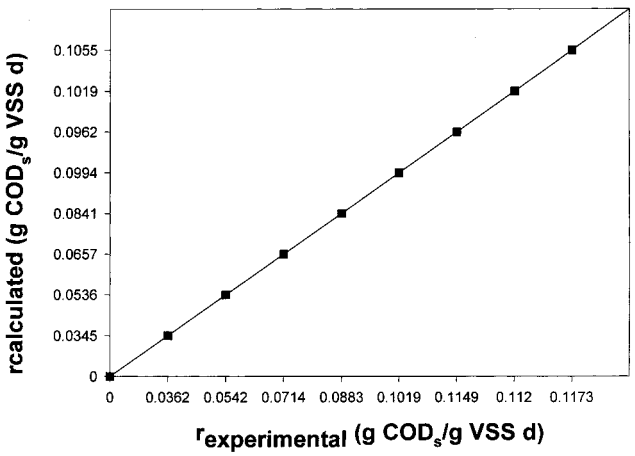

Figure 6. Comparison between the experimental specific substrate utilization rate values and those theoretical predicted by eq 5.

LITERATURE CITED

(1) Zeeman, G.; Sutter, K.; Vens, T.; Koster, J. M.; Wellinger, A. Psychrophilic digestion of dairy cattle and pig manure. Start-up procedure of batch, fed-batch and CSTR type digesters. *Biol. Wastes* **1988**, *26*, 15−31.

(2) Safley, L. M., Jr.; Westerman, P. W. Psychrophilic anaerobic digestion of animal manure, proposed design methodology. *Biol. Wastes* **1990**, *34*, 133−148.

(3) Meher, K. K.; Murthy, M. V. S.; Gollakota, K. G. Psychrophilic anaerobic digestion of human waste. *Bioresour. Technol.* **1994**, *50*, 103−106.

(4) Rebac, S.; Ruskova, J.; Gerbens, S.; van Lier, J. B.; Stams, A. J. M.; Lettinga, G. High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Ferment. Bioeng.* **1995**, *80*, 499−506.

(5) Lokshina, L. Y.; Vavilin, V. A. Kinetic analysis of the key stages of low-temperature methanogenesis. *Ecol. Modell.* **1999**, *117*, 285−303.

(6) Kotsyurbenko, O. R.; Nozhevnikova, A. N.; Soloviova, T. I.; Zavarzin, G. A. Methanogenesis at low temperatures by microflora of tundra wetland soil. *Antonie van Leeuwenhoek* **1996**, *69*, 75−86.

(7) Kusel, K.; Drake, H. L. Acetate synthesis in soil from a Bavarian beech forest. *Appl. Environ. Microbiol.* **1994**, *60*, 1370−1373.

(8) Kusel, K.; Drake, H. L. Effect of environmental parameters on the formation and turnover of acetate by forest soils. *Appl. Environ. Microbiol.* **1995**, *61*, 3667−3675.

(9) Kusel, K.; Drake, H. L. Anaerobic capacities of leaf litter. *Appl. Environ. Microbiol.* **1996**, *62*, 4216−4219.

(10) Wagner, C.; Griebhammer, A.; Drake, H. L. Acetogenic capacities and the anaerobic turnover of carbon in a Kansas prairie soil. *Appl. Environ. Microbiol.* **1996**, *62*, 494−500.

(11) Kotsyurbenko, O. R.; Nozhevnikova, A. N.; Zavarzin, G. A. Anaerobic degradation of organic matter by psychrophilic microorganisms. *J. Gen. Biol.* **1992**, *53*, 159−175.

(12) Kotsyurbenko, O. R.; Nozhevnikova, A. N.; Zavarzin, G. A. Methanogenic degradation of organic matter by anaerobic bacteria at low temperature. *Chemosphere* **1993**, *62*, 1745−1761.

(13) Schultz, S.; Conrad, R. Influence of temperature on pathways to methane production in the permanently cold profundal sediment of Lake Constance. *FEMS Microbiol. Ecol.* **1996**, *20*, 1−14.

(14) Nozhevnikova, A. N.; Holliger, C.; Amman, A.; Zehnder, A. J. B. Psychrophilic methanogenesis in sediments of deep lakes. In *Proceedings of the International Conference on Anaerobic Digestion*, Vol. 2; Noike, T., Tilche, A., Hanaki, K., Eds.; Elsevier Science Japan: Sendai, Japan, 1997; pp 414−421.

(15) Kotsyurbenko, O. R.; Nozhevnikova, A. N.; Kalyuzhnyi, S. V.; Zavarzin, G. A. Methanogenic digestion of cattle manure at low temperatures. *Mikrobiologia* **1993**, *62*, 761−771.

(16) Parshina, S. N.; Nozhevnikova, A. N.; Kalyuzhnyi, S. V. Fermentation of protein substrates by pig's manure microflora. *Mikrobiologia* **1993**, *62*, 169−180.
(17) Borja, R.; González, E.; Raposo, F.; Millán, F.; Martín, A. Performance evaluation of a mesophilic anaerobic fluidised reactor treating wastewater derived from the production of proteins from extracted sunflower flour. *Bioresour. Technol.* **2001**, *76*, 45−52.
(18) Borja, R.; Banks, C. J.; Wang, Z. Effect of organic loading rate on anaerobic treatment of slaughterhouse wastewater in a fluidised-bed reactor. *Bioresour. Technol.* **1995**, *52*, 157−162.
(19) Maestrojuan, G. M.; Fiestas, J. A. A study of the behaviour of anaerobic bacteria in the presence of diverse materials usable as support. In *Proceedings of the Fifth International Symposium on Anaerobic Digestion;* Tilche, A., Rozzi, A., Eds.; Monduzzi Editore, S.p.A.: Bologna, Italy, 1988; pp 129−132.
(20) Pérez-Rodriguez, J. L.; Carretero, M. I.; Maqueda, C. Behaviour of sepiolite, vermiculite and montmorillonite as supports in anaerobic digesters. *Appl. Clay Sci.* **1989**, *4*, 69−82.
(21) Borja, R.; Banks, C. J. Kinetics of methane production from palm oil mill effluent in an immobilised cell bioreactor using saponite as support medium. *Bioresour. Technol.* **1994**, *48*, 209−214.
(22) Borja, R.; Banks, C. J. Kinetics of an anaerobic fluidised-bed system used for the purification of fruit processing wastewater. *Chem. Eng. J.; Biochem. Eng. J.* **1994**, *54*, B25−B32.
(23) Borja, R.; Banks, C. J. Response of an anaerobic fluidised-bed reactor treating ice-cream wastewater to organic, hydraulic, temperature and pH shocks. *J. Biotechnol.* **1995**, *39*, 251−259.
(24) Fiestas, J. A.; Martín, A.; Borja, R. Influence of immobilisation supports on the kinetic constants of anaerobic purification of olive mill wastewater. *Biol. Wastes* **1990**, *33*, 131−142.
(25) Borja, R.; González, E.; Raposo, F.; Millán, F.; Martín, A.; Sánchez, E. Depuración anaerobia psicrófila de las aguas residuales procedentes de la obtención de aislados proteicos a partir de harina de girasol. *Revista Técnica de Medio Ambiente (RETEMA)*, **2001**, *14*, 17−23.
(26) Chen, S. J.; Li, C. T.; Shieh, W. K. Evaluation of the anaerobic fluidised-bed system. Part III. Biomass hold-up and characteristics. *J. Chem. Technol. Biotechnol.* **1985**, *35B*, 183−190.
(27) American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater*, 17th ed.; APHA: Washington DC, 1989.
(28) Fannin, K. F. Start-up, operation, stability and control. In *Anaerobic Digestion of Biomass*; Chynoweth, D. P., Isaacson, R., Eds.; Elsevier: London, U.K., 1987; pp 171−196.
(29) Wheatley, A. *Anaerobic Digestion: A Waste Treatment Technology*. SCI, Elsevier: London, U.K., 1990.
(30) Lawrence, A. W.; McCarty, P. L. Kinetics of methane fermentation in anaerobic treatment. *J. Water Pollut. Control Fed.* **1969**, *41*, R1−16.
(31) Stronach, S. M.; Rudd, T.; Lester, J. N. *Anaerobic Digestion Processes in Industrial Wastewater Treatment.* Springer-Verlag: Berlin (Germany), 1986.
(32) Borja, R.; Banks, C. J. Kinetics of an up-flow anaerobic sludge blanket reactor treating ice-cream wastewater. *Environ. Technol.* **1994**, *15*, 219−232.
(33) Borja, R.; González, E.; Raposo, F.; Millán, F.; Martín, A. Assessment of kinetic and macroenergetic parameters for a mesophilic anaerobic fluidised-bed reactor treating wastewater derived from the production of protein isolates from extracted sunflower flour. *Process Biochem.* **2000**, *36*, 369−375.

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