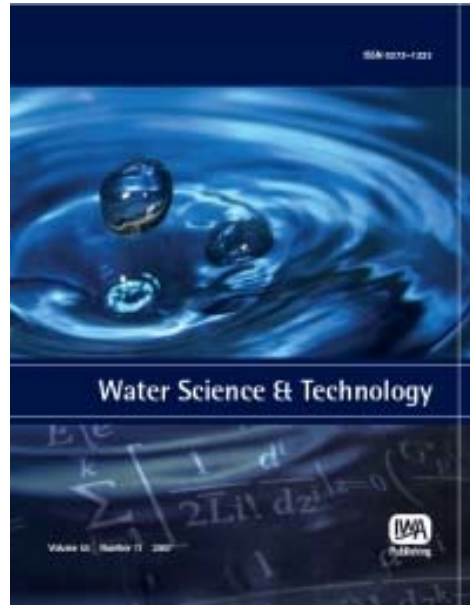


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Influence of temperature on the hydrolysis, acidogenesis and methanogenesis in mesophilic anaerobic digestion: parameter identification and modeling application

A. Donoso-Bravo, C. Retamal, M. Carballa, G. Ruiz-Filippi and R. Chamy

ABSTRACT

The effect of temperature on the kinetic parameters involved in the main reactions of the anaerobic digestion process was studied. Batch tests with starch, glucose and acetic acid as substrates for hydrolysis, acidogenesis and methanogenesis, respectively, were performed in a temperature range between 15 and 45°C. First order kinetics was assumed to determine the hydrolysis rate constant, while Monod and Haldane kinetics were considered for acidogenesis and methanogenesis, respectively. The results obtained showed that the anaerobic process is strongly influenced by temperature, with acidogenesis exerting the highest effect. The Cardinal Temperature Model 1 with an inflection point (CTM1) fitted properly the experimental data in the whole temperature range, except for the maximum degradation rate of acidogenesis. A simple case-study assessing the effect of temperature on an anaerobic CSTR performance indicated that with relatively simple substrates, like starch, the limiting reaction would change depending on temperature. However, when more complex substrates are used (e.g. sewage sludge), the hydrolysis might become more quickly into the limiting step.

Key words | acidogenesis, anaerobic digestion, hydrolysis, modeling, temperature

A. Donoso-Bravo (corresponding author)

C. Retamal

M. Carballa

G. Ruiz-Filippi

R. Chamy

School of Biochemical Engineering,
Pontificia Universidad Católica de Valparaíso,
General Cruz 34,
Valparaíso,
Chile
E-mail: andres.donosob@mail.ucv.cl

M. Carballa

Department of Chemical Engineering,
School of Engineering,
University of Santiago de Compostela,
Rúa Lope Gómez de Marzoa s/n,
15782 Santiago de Compostela
Spain

INTRODUCTION

Nowadays, anaerobic digestion might be considered as a consolidated technology with more than 2200 high-rate reactors implemented worldwide, treating different types of wastes and wastewaters coming from different sectors, such as agro-food industry, beverage, alcohol distillery and pulp and paper industries (van Lier 2008).

However, there are a number of factors which affect the anaerobic digestion process, including the substrate characteristics, the reactor configuration, the operational parameters, such as hydraulic retention time (HRT), solids retention time (SRT) and organic loading rate (OLR), and the environmental factors like temperature and pH (Banerjee *et al.* 1998).

As a complex multi-step process, the overall kinetics of waste utilization during anaerobic treatment is governed by the kinetics of the slowest step, which often corresponds to

the methanization process. On the contrary, when treating complex organic matter or anaerobic treatment is carried out at low temperature, hydrolysis is often considered as the limiting step (Vavilin *et al.* 2008).

The temperature at which the anaerobic digestion occurs can significantly affect the conversion, kinetics, stability, effluent quality, and consequently, the methane yield of the process (Sanchez *et al.* 2001). Microorganisms are generally divided into three thermal groups, psychrophiles, mesophiles and thermophiles, with optimum temperatures below 20°C, 25–40°C and higher than 45°C, respectively (van Lier *et al.* 1997), and it has been demonstrated that the anaerobic degradation rate of organic matter increases with temperature when psychrophilic, mesophilic and thermophilic conditions are compared (Sanchez *et al.* 2001). However, anaerobic

digestion has been traditionally performed in mesophilic range (35–37°C) despite the temperature of certain wastewaters might be either warmer or cooler. Treating these wastewaters at their natural temperatures would often be beneficial because of the reduced resources and costs (Kettunen & Rintala 1997).

The influence of temperature has been extensively studied on the rate-limiting methanogenic phase at both mesophilic and thermophilic conditions (Hegde & Pullammanappallil 2007). However, little attention has been paid on the effect of temperature on hydrolysis and acidogenesis. Hydrolysis rate constants are highly dependent on temperature since hydrolysis is a biochemical reaction catalyzed by enzymes, which are very thermally sensitive (Sanders *et al.* 2000). Moreover, a better understanding of temperature effects on acidogenesis can result in the improvement of digester stability due to physical separation of phases, the increase in the concentration of soluble organics and the optimization of biological nutrient removal processes (Banerjee *et al.* 1998). In addition, few studies have been carried out on the effect of temperature on the overall process of anaerobic digestion. Banik *et al.* (1998) studied the effect of temperature on the overall kinetic parameters of the anaerobic biomass but just up to 25°C. Since temperature variations may not have the same effect on the different stages of anaerobic digestion (hydrolysis, acidogenesis and methanogenesis), more knowledge is required to identify and select corrective actions for temperature disturbances on anaerobic reactors.

The objective of this study was to determine the kinetic parameters that characterize hydrolysis, acidogenesis and methanogenic stages at different temperatures in order to develop a mathematical model describing the influence of temperature on each single stage.

MATERIALS AND METHODS

Experimental set-up

Batch experiments were run in glass serum bottles with a total liquid volume of 200, 250 and 100 mL for hydrolysis, acidogenesis and methanogenesis assays, respectively. Macronutrients, micronutrients and yeast

extract were added according to Field *et al.* (1988). Starch (1.0–3.0 g/L), glucose (0.1–1.0 g/L) and acetic acid (0.1–25.0 g/L) were used for hydrolysis, acidogenesis and methanogenesis assays, respectively. Several temperatures were tested for the different stages, i.e. hydrolysis (12, 22, 30, 37 and 45°C), acidogenesis (12, 22, 30, 37, 42 and 45°C) and methanogenesis (12, 25, 37 and 45°C). Sodium bicarbonate was used as buffer at a concentration of 1 g/gCOD_{added} (Soto *et al.* 1993) in the hydrolysis assays, while for acidogenesis and methanogenesis assays, phosphate was used at concentrations ranging from 0.007–0.070 M and 0.009 M, respectively (Retamal 2008).

The temperature of the experiments was maintained by using a thermostatic water bath. The bottles were inoculated with anaerobic biomass coming from a mesophilic sewage sludge digester at a final concentration of 1.03 ± 0.14 gVSS/L. Different substrate concentrations were tested in duplicate or triplicate at each temperature and the substrate consumption over time, pH and suspended solids concentration at the end of the experiments were the parameters monitored.

For acidogenesis and methanogenesis, a biomass linear range study was performed at 37°C with glucose (0.09 g/L) and acetic acid (0.12 g/L), respectively. For acidogenesis, three biomass concentrations, 0.20, 0.54 and 1.07 gVSS/L, were used and the volumetric consumption rates of glucose were calculated. The glucose consumption rate at 0.54 gVSS/L was double than the value obtained at 0.20 gVSS/L, while no differences were observed between 0.54 and 1.07 gVSS/L. Hence, a biomass concentration of 0.54 g/L was selected. For methanogenesis, two biomass concentrations, 0.32 and 0.65 gVSS/L, were used and the volumetric consumption rates of acetic acid were $9.6 \cdot 10^{-3}$ and $1.9 \cdot 10^{-2}$ g/L-d, respectively. Thus, a biomass concentration of 0.4 gVSS/L was used.

Determination of kinetic parameters

Kinetic equations

First order kinetics was considered for the hydrolysis of particulate organic matter (Equation 1), while Monod-type (Equation 2) and Haldane-type (Equation 3) kinetics were assumed for acidogenesis and methanogenesis

(Bernard *et al.* 2001).

$$v_h = -k_h \cdot S_h \quad (1)$$

$$v_1 = v_{\max 1} \frac{S_1}{K_{S_1} + S_1} \quad (2)$$

$$v_2 = v_{\max 2} \frac{S_2}{K_{S_2} + S_2 + \frac{S_2^2}{K_I}} \quad (3)$$

where v_h is the hydrolysis reaction rate (g/L-d), S_h is the hydrolysis substrate concentration (g/L), k_h is the hydrolysis rate constant (d⁻¹), v_1 is the acidogenesis reaction rate (g/gVSS-d), S_1 is the acidogenesis substrate concentration (g/L), $v_{\max 1}$ is the maximum degradation rate for acidogenesis (g/gVSS-d), K_{S_1} is the half saturation constant for acidogenesis (g/L), v_2 is the methanogenesis reaction rate (g/gVSS-d), S_2 is the methanogenesis substrate concentration (g/L), $v_{\max 2}$ is the maximum degradation rate for methanogenesis (g/gVSS-d), K_{S_2} is the half saturation constant for methanogenesis (g/L) and K_I is the inhibition constant associated with substrate S_2 .

Parameters estimation

The hydrolysis rate constant is obtained from Equation (1), by representing $\ln S_{0h}/S_h$ versus time (slope). The determi-

Finally, a nonlinear optimization by least squares procedure is applied to calculate the unknown parameters by minimizing a cost function (Equation 6), which measures the difference between the experimental measurements and the corresponding simulated value (the values obtained with the linearization method are used as initial values in the simulation process).

$$J(\psi) = \min \sum_{t=1}^N (v_m(t) - v(t, \psi))^2 \Big|_{\psi_0 = \psi_L} \quad (6)$$

where v_m is the velocity consumption obtained for measurements, v is the corresponding simulated velocity and N is the number of measurements.

Models of temperature effect

The influence of temperature on the hydrolysis constant rate has been often described by the Arrhenius model. However, this model is limited by a maximum value of temperature above which the temperature effect can not be further evaluated. In contrast, the Cardinal Temperature Model 1 with an inflection point (CTM1) proposed by Rosso *et al.* (1993) is able to model all temperatures tested (Equation 7).

$$b = b_{\text{opt}} \frac{(T - T_{\max})(T - T_{\min})^2}{(T_{\text{opt}} - T_{\min})[(T_{\text{opt}} - T_{\min}) \cdot (T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max}) \cdot (T_{\text{opt}} + T_{\min} - 2T)]} \quad (7)$$

nation of the kinetic parameters for acidogenesis and methanogenesis was carried out from the initial consumption rate (Retamal 2008) by using the linearization Lineweaver-Burke method (Equations (4) and (5)). Since the initial concentration of biomass may exert an important effect on the biodegradation kinetics (Urrea *et al.* 2008), these assays were performed within the biomass lineal range.

$$\frac{1}{v_1} = \frac{K_{S_1}}{Vm_1} \cdot \frac{1}{S_1} + \frac{1}{Vm_1} \quad (4)$$

$$\frac{1}{v_2} = \frac{K_{S_2}}{Vm_2} \cdot \frac{1}{S_2} + \frac{1}{Vm_2} + \frac{S_2}{Vm_2 \cdot K_I} \quad (5)$$

where $\psi_L = (K_{S_1}, K_{S_2}, K_I, Vm_1, Vm_2)$ are the unknown parameters.

where T_{\min} , T_{opt} and T_{\max} were the minimum, optimum and maximum temperatures, respectively (°C), and b_{opt} is the optimum value of the kinetic parameter under study. All these parameters were obtained from a nonlinear optimization by least squares procedure as described previously. Figure 1 summarizes all the calculations procedure.

Analytical methods

Starch concentrations were determined as the difference between the total sugar concentrations (Dubois *et al.* 1956) and the reducing sugar concentrations, i.e. glucose concentrations (Miller 1959). pH, Volatile Suspended Solids (VSS) and Volatile Fatty Acids (VFA) were determined according to standard methods (APHA 1995).

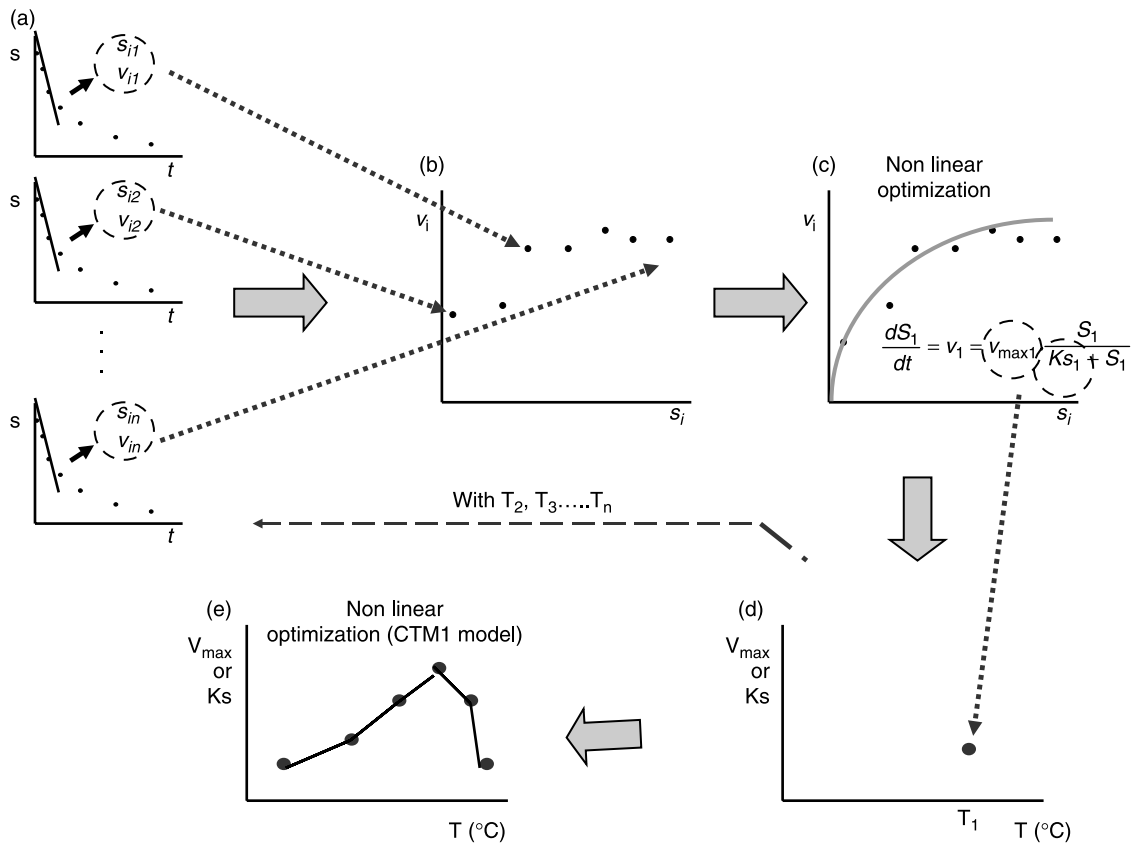


Figure 1 | Calculation procedure for the determination of the kinetic parameters and the evaluation of temperature influence. Case-study: acidogenesis. (A) Initial rates calculation; (B) kinetic-profile construction; (C) model fit; (D) temperature influence; and, (E) temperature-model fit.

RESULTS AND DISCUSSION

Hydrolysis rate constants as a function of temperature

Figure 2a shows the hydrolysis rate constants of starch at the different temperatures tested. It can be observed that the hydrolysis rate constants increased with temperature up to an optimum value of $21.1 \pm 2.2 \text{ d}^{-1}$ at 37°C . However, a lower value was obtained at 45°C ($7.9 \pm 1.7 \text{ d}^{-1}$), which is probably due to the fact that this temperature is in between the optimum values for mesophilic and thermophilic conditions. In the temperature range of 12 to 37°C , the hydrolysis rate constants fitted well the Arrhenius equation and a value of 72 kJ/mol for the activation energy was calculated, which is a typical value for enzymatic kinetics under anaerobic conditions (Veeken & Hamelers 1999). On the contrary, the CTM1 model is more appropriate

to describe the effect of temperature over the whole temperature range tested. The parameters of CTM1 are shown in Table 1. It can be observed that the optimum modeled temperature for hydrolysis (40.3°C) is lower than the value obtained in the experiments (37°C), while the optimum modeled hydrolysis rate constant (22.8 d^{-1}) is higher than the experimental value (21.1 d^{-1}).

The values obtained for the hydrolysis constant in this study were largely greater than other values reported in literature (Siegrist *et al.* 2002; Vavilin *et al.* 2008). This fact is probably explained by the use of starch as substrate, which is one of the most readily hydrolysable substrates in contrast to the more complex substrates, such as sludge or lipids, used in other literature studies. With regard to the hydrolysis constant dependency on temperature, Siegrist *et al.* (2002) also found that this parameter is strongly influenced by temperature.

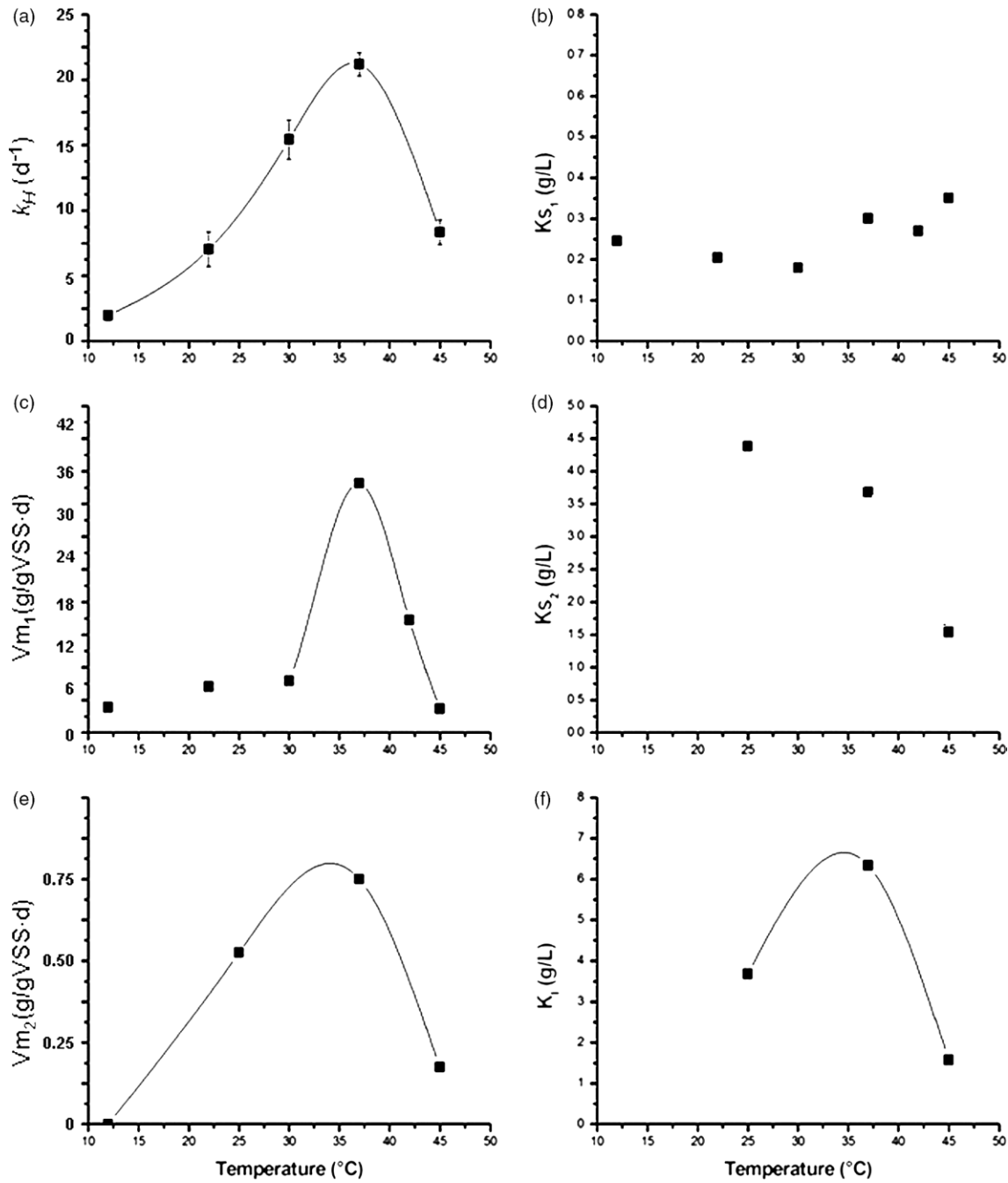


Figure 2 | (a) Hydrolysis rate constants in function of temperature using starch as substrate; (b) Maximum degradation rates of acidogenesis in function of temperature using glucose as substrate; (c) Maximum degradation rates of methanogenesis using acetic as substrate; (d) Influence of temperature on half saturation constant in acidogenesis stage; (e) Influence of temperature on half saturation constant in methanogenesis stage; (f) Influence of temperature inhibition constant in methanogenesis stage. ■, experimental data; —, CTM1 fit.

Table 1 | CTM1 parameters for optimum degradation rates during hydrolysis, acidogenesis and methanogenesis using starch, glucose and acetic acid as substrates, respectively

Parameter		T_{\min} (°C)	T_{opt} (°C)	T_{\max} (°C)	$k_{H\text{opt}}$ (d ⁻¹)	$Vm_{1\text{opt}}$ (g/gVSS-d)	$Vm_{2\text{opt}}$ (g/gVSS-d)	$K_{I\text{opt}}$ (g/L)
Hydrolysis	k_h	4.2	40.3	45.5	22.8	–	–	–
Acidogenesis	Vm_1	28.9	37.0	45.2	–	33.6	–	–
Methanogenesis	Vm_2	11.1	34.1	46.3	–	–	0.72	–
	K_I	11.9	34.3	46.2	–	–	–	6.45

Influence of temperature on acidogenesis and methanogenesis

Maximum degradation rates

Figure 2b shows the influence of temperature on the maximum degradation rate during acidogenesis. Whereas there was almost no difference between 12 and 30°C, a significant increase was observed at 37°C with again a sharp decrease above 40°C. In this case, only the temperature range between 30 and 45°C was considered for CTM1 modeling, which showed that the optimum temperature for acidogenesis is lower than that for hydrolysis (Table 1).

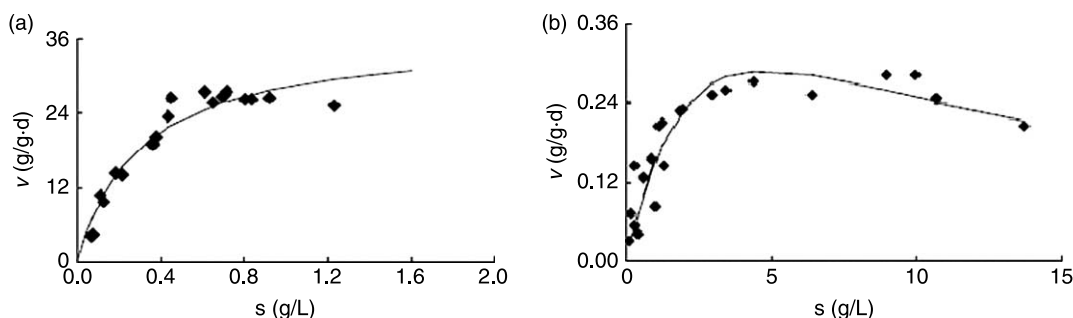
Figure 2c shows the influence of temperature on the maximum degradation rate during methanogenesis and the CTM1 fit over the whole temperature range tested. It can be observed that the maximum degradation rate during methanogenesis was negligible below 15°C and it increased progressively with temperature up to 30–35°C, with lower values again at higher temperatures. CTM1 model indicates that the optimum temperature for methanogenesis (34.1°C) is lower than for hydrolysis and acidogenesis (Table 1).

Overall, the three reactions diminished their activity as the temperature decreased, as also observed by Banik *et al.* (1998). However, the impact of temperature decrease is different for each stage. Assuming 37°C as the operational

temperature of an anaerobic reactor, a 5°C decrease would result in 50 and 10% slower kinetics of acidogenesis and hydrolysis, respectively, with almost no effect on methanogenesis.

Affinity and inhibition constant

Figure 2b and Figure 2d show the influence of temperature on glucose (acidogenesis) and acetic acid (methanogenesis) affinity constants. Similar values were obtained by Siegrist *et al.* (2002), but these authors also found an increase of these parameters at thermophilic conditions (55°C), while in the present study, no significant variations were observed for affinity constant of glucose in the temperature range tested. This finding was also obtained by Banik *et al.* (1998) for the global affinity constant of anaerobic population. However, the half saturation constant of acetic acid decreased from 4.25 g/L at 25°C to 1.5 g/L at 45°C. The assay carried out at 12°C did not produce methane during the 3 months of experiment, thus not being possible the calculation of this parameter at this low temperature. Concerning the inhibition constant of acetic acid (Figure 2f), the influence of temperature was similar to that on the maximum degradation rate (Figure 2e) and it could be modeled by CTM1 (Table 1).

**Figure 3** | Profiles of the initial reaction rate at different substrates concentrations at 37°C for acidogenesis (a) and methanogenesis (b).

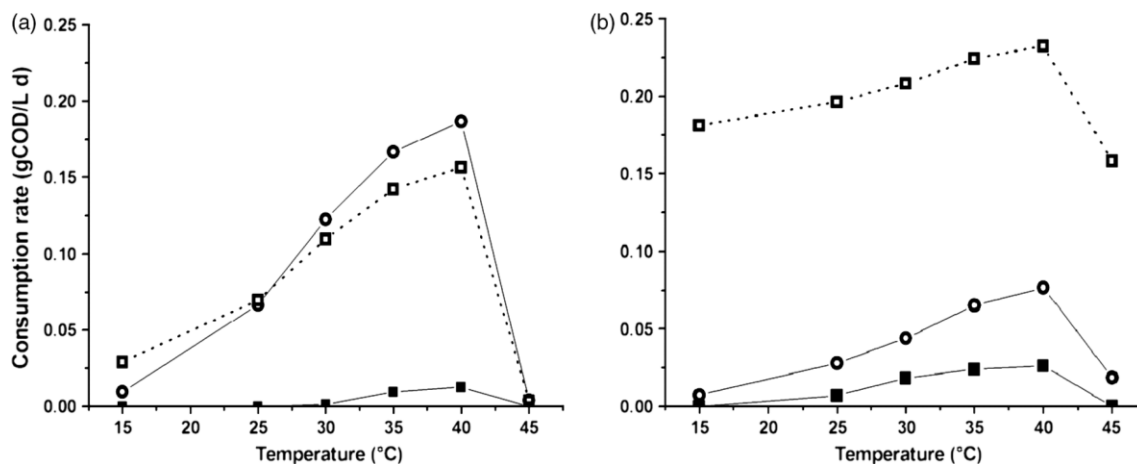


Figure 4 | Steady state COD consumption rate during (○) hydrolysis, (□) acidogenesis and (■) methanogenesis between 15 and 45°C. (a) starch-wastewater (b) sludge.

Dynamic behavior

Figure 3 shows the dynamic behavior of the consumption rate at different concentration of substrate for acidogenesis (Figure 3a) and methanogenesis (Figure 3b) at one temperature. It can be observed that the reaction behaves according to a Monod and Haldane model.

Case-study: continuous stirred tank reactor (CSTR)

A two-population and three-reaction model (Donoso-Bravo 2008) adapted from the two-reaction model developed by Bernard *et al.* (2001) was used to evaluate the influence of temperature on the performance of an anaerobic CSTR. A starch-wastewater containing 5.0 gCOD/L and sludge from an activated sludge plant were considered. A hydraulic

retention time (HRT) of 20 d and six temperatures (15, 25, 30, 35, 40 and 45°C) were assumed for the simulation. For each temperature, the kinetic parameters were calculated from the results obtained in the previous experiments in the case of starch-wastewater. For the anaerobic sludge treatment, a hydrolysis constant value of 0.016 d^{-1} (which is three orders of magnitude lower than that of starch) determined by Retamal (2008) at 37°C was used, assuming a CTM1 behavior for the same range of temperature. In both cases, a 90% of particulate organic fraction was assumed.

The results of the variables were obtained at steady state and they were considered as the initial conditions of the system for the next run. The system was run in total for 500 days, and the results are shown in Figure 4.

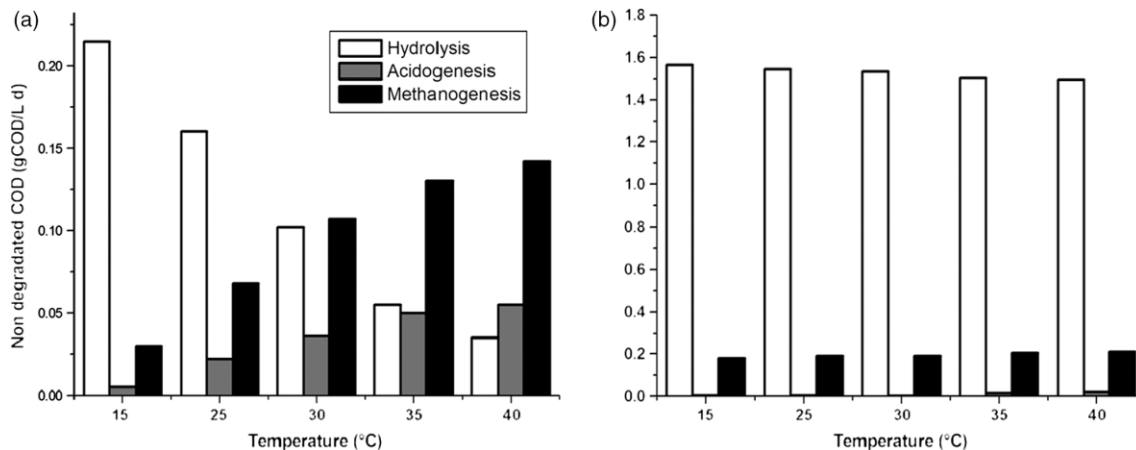


Figure 5 | Non-degraded COD accumulation during hydrolysis, acidogenesis and methanogenesis at different temperatures. (a) starch-wastewater (b) sludge.

In general terms, it can be noted that the methanogenic reaction rate is the lowest for the temperature range studied and for the kinetic parameters determined. However, with sludge (Figure 4b), the hydrolysis and the methanogenesis rates are closer than with starch, which shows that the nature of the substrate has a significant influence on the overall process.

In order to evaluate the limiting step at different temperatures, a model was used to determine the non-degraded COD in each phase (Figure 5). Figure 5a shows that, for starch-wastewater, an important accumulation of particulate COD would occur below 30°C, whereas at higher temperatures the methanogenic reaction would turn into the limiting step. For sludge (Figure 5b), hydrolysis remains as the limiting step for all studied temperatures. That is the reason why the increasing use of sludge-pretreatment methods prior to anaerobic digestion to diminish the particulate organic fraction of the sludge, and thus increasing the overall rate of the anaerobic digestion.

CONCLUSIONS

Knowledge about the effect of temperature on the kinetic parameters of the main anaerobic reactions represents an important progress in the anaerobic digestion field. This work shows that the anaerobic process is strongly influenced by temperature, with acidogenesis showing the highest effect. Therefore, it can be concluded that this stage plays a key role in the stability of the overall anaerobic process, since a small change in the operational temperature (around 5°C), results in a decrease of 50% in the acidogenesis reaction rate, affecting consequently the methanogenesis stage.

Moreover, CTM1 modeled properly the influence of temperature on the different kinetic parameters involved in anaerobic process in the temperature range tested, except for the maximum degradation rate of acidogenesis, which could be only modeled between 30 and 45°C. The simple case-study to assess the effect of temperature on an anaerobic CSTR performance indicated that with relatively simple substrates, like starch, the limiting reaction would change depending on temperature. However, when more

complex substrates are used (e.g. sewage sludge), the hydrolysis was clearly the limiting step.

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