# Effect of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal in the batch anaerobic digestion process of cattle manure

**E. Sánchez, R. Borja, P. Weiland, L. Travieso, A. Martín**

**Abstract** A study of the effect of temperature and pH on the kinetics of methane production and organic nitrogen and phosphorus degradation in the anaerobic digestion process of cattle manure was carried out. Two laboratory-scale batch completely mixed reactors, operating at 35 °C (mesophilic temperature), and other two, operating at 60 °C (thermophilic temperature) were used. For each temperature selected, the influent pH values were 7.6 (initial pH of the waste used) and 7.0. The apparent kinetic constants of the biomethanization process increased 2.3 times when the initial pH of the influent was increased from 7.0 to 7.6 at mesophilic temperature. The values found at thermophilic temperature were similar. The kinetic constants of methane production decreased 2.6 and 7.2 times when the operating temperature increased from 35 °C to 60 °C for the experiments carried out at initial pH of 7.0 and 7.6, respectively. The methane yield coefficient (l $CH_4$ STP/g VS removed) also decreased when the temperature increased from 35 °C to 60 °C for the two initial pH values studied. This behaviour agreed with the major inhibition level observed at thermophilic temperature as a result of the higher organic nitrogen removal and ammonia nitrogen production observed at 60 °C. Specifically, the specific rate constants for organic nitrogen removal and ammonia nitrogen production increased 3.6 and 12 times when the temperature was increased from 35 °C to 60 °C for the experiments carried out at initial pH values of 7.0 and 7.6, respectively. In the same way, the values of the kinetic constant for phosphorus removal were 44% and 80% higher than those obtained at 35 °C for the two initial pH values above-mentioned, respectively. Finally, the experimental values of organic nitrogen and phosphorus concentrations were reproduced with deviations equal to or less than 10% and 15% in every case, respectively.

## List of Symbols

| | |
|---|---|
| A.N. | Ammonia nitrogen (mg/l) |
| O.N. | Organic nitrogen (mg/l) |
| $Q_g$ | Biogas production (l) |
| $Q_{methane}$ | Methane production (l) |
| STP | Standard temperature and pressure conditions |
| VS | Volatile solids (g/l) |

E. Sánchez
Consultores Ambientales (CONAM). Calle 14,
n° 308, entre 3ª y 5ª Avda. La Habana, Cuba

R. Borja (✉)
Instituto de la Grasa (C.S.I.C.). Avda. Padre García Tejero 4,
41012 Sevilla, Spain

P. Weiland
Institute of Technology,
Federal Research Center of Agriculture (FAL),
Bundesallee 50, D-38116 Braunschweig, Germany

L. Travieso
Departamento de Estudios sobre Contaminación Ambiental
(DECA-CNIC),
Centro Nacional de Investigaciones Científicas (CNIC),
P.O. Box 6990, La Habana, Cuba

A. Martín
Departamento de Ingeniería Química, Facultad de Ciencias,
Avda. San Alberto Magno s/n, 14004 Córdoba, Spain

## 1
## Introduction

The presence of nutrients in final effluents of waste treatment plants constitutes a serious damage on the environment due to the eutrophization of surface water and the pollution of underground sources of water affecting the further utilization of these waters for human consumption [1].

The main sources of nutrients are originated in animal wastes. Among them, cattle manure is considered to be one of the most important sources of nutrients (nitrogen and phosphorus). In dairy cattle, only 25% of the nitrogen consumed is retained by the animals. The remainder is excreted with an estimated 52% in the urine and 48% in the feces [2, 3]. Besides of this, phosphorus constitutes about 1.1–1.3% of the total solids concentration of cattle manure. Both nitrogen and phosphorus compounds must be reduced in the effluents before the final disposal. In order to obtain chemical species of nutrients able to be economically removable, organic nitrogen must be transformed to ammonia nitrogen and organic phosphorus and polyphosphates must be transformed to orthophosphate and further to metallic forms easily removable by sedimentation [4, 5].

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As a consequence of anaerobic digestion, proteins, aminoacids and urea are transformed to ammonia nitrogen and organic phosphorus and polyphosphate and finally orthophosphate are transformed to metallic phosphorus by biological reduction or assimilated by the microorganisms [4–6].

Temperature and pH play an important role on the anaerobic digestion, hence, on the biotransformation of nitrogen and phosphorus organic compounds [6, 7]. During the hydrolytic and acetogenic steps (initial stages of the anaerobic digestion) organic nitrogen compounds are degraded and ammonia nitrogen is released at different rates depending on the molecular complexity of the compounds. For instance urea is degraded faster than proteins [8]. Organic phosphorus compounds and polyphosphates are decomposed to simple inorganic compounds and partially assimilated by the microorganisms for the synthesis [4–6, 9, 10].

Cattle manure is a complex substrate containing undissolved and dissolved organic matter such as polysaccharides, lipids, proteins and inorganic compounds of importance for the chemical environment [11].

An increase of temperature provokes an increase of the rate of hydrolysis, acidification and methanogenesis stages under certain conditions contributing to accelerate the organic nitrogen and phosphorus transformations. A decrease of pH determines also an increase of organic matter hydrolysis which is favourable for organic nitrogen and phosphorus decomposition [12, 13].

In anaerobic digestion of cattle manure the increase of pH could affect the biotransformation of organic nitrogen and phosphorus. The pH could increase during the process when ammonia nitrogen is accumulated and inhibition could appear due to the presence of free ammonia, which is produced at around pH 8.0 [14–16]. In order to obtain a maximum conversion of organic nitrogen and phosphorus, the pH and temperature of the process must be taken into account [16–18].

The aim of this work was to evaluate the effect of temperature and pH on the kinetics of methane production and organic nitrogen and phosphorus removal in the anaerobic digestion process of cattle manure.

# 2
# Materials and methods

## 2.1
## Waste
Previous to the experiments cattle manure was screened by a 2 mm sieve. The waste was characterized by the determination of total and volatile solids, organic and ammonia nitrogen, orthophosphate and pH using standard methods [19].

Table 1 shows the mean values and variance coefficients of 20 samples of the cattle manure used.

## 2.2
## Equipment
The anaerobic digesters consisted of four acrylic plastic cylinders of 3 litres of effective volume. The cylinders were hermetically closed using plastic covers with rubber joints

**Table 1.** Characteristics of the cattle manure used during the experiments

| Parameter | Mean value | Variance coefficient (%) |
|---|---|---|
| Total solids (g/l) | 40.3 | 7 |
| Volatile solids (g/l) | 25.4 | 10 |
| Organic nitrogen (mg/l) | 3030 | 12 |
| Ammonia nitrogen (mg/l) | 1471 | 14 |
| Orthophosphate (mg/l) | 816 | 13 |
| pH | 7.6 | 10 |

provided with two connections, one for biogas outlet and the other for liquor sampling. The reactor contents were mixed by magnetic stirrers at 100 rpm. Biogas was accumulated in plastic bags for volume and composition determination. Biogas volume was corrected at standard temperature and pressure conditions. The experiments were carried out at 35 °C (mesophilic temperature) and 60 °C (thermophilic temperature). The temperatures were maintained at 35 °C and 60 °C by standing the digesters in water baths.

## 2.3
## Experimental procedure
Two batch reactors of 3 litres volume were used for the experiment at mesophilic temperature (35 °C) and other two for the thermophilic one (60 °C). At both mesophilic and thermophilic temperatures, one of the reactors initiated the experiment at pH 7.0 and the second one was used as control (initial pH 7.6). Concentrated HCl (5 M) was used to reduce the initial pH of the waste to the value previously selected.

The reactors were inoculated with 600 ml of well digested cattle anaerobic sludge (180 days digestion time) with a total solid concentration of 4.5% and volatile solids of 58.3% on a dry basis. Before the experiments were started, feed volumes between 10 and 220 ml of diluted cattle manure (10%) at the corresponding operating pH and temperature, were added daily during a three week period until the working volumes of the reactors were completed.

Previous to the beginning of the experiments a volume of 2600 ml of each reactor content was taken out after a settlement period of 8 hours which was sufficient for separation and retention of the biomass. After that, the same volume (2600 ml) of fresh cattle manure (Table 1) was added to each reactor at the pH and temperature conditions above-mentioned.

Each experimental run was carried out in triplicate during a digestion time of 33 days. As the deviations between replicate samples were always less than 5%, mean values are reported (Tables 2–5). Samples were taken each four days except for the last one taken at day 33. The following parameters were analyzed following the recommendations of standard methods [19]: volatile solids (VS), organic (O.N.) and ammonia nitrogen (A.N.), orthophosphate ($PO_4^{3-}$) and pH. Methane concentration and biogas volumes were measured at the same time of liquid samples by means of an infrared detector (Siemens).

**Table 2.** Results obtained for the experimental run corresponding to 35 °C and pH 7.6

| Time (days) | $Q_g$ (l) | $Q_{methane}$ (l) | VS (g/l) | O.N. (mg/l) | A.N. (mg/l) | $PO_4^{3-}$ (mg/l) | pH |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 25.41 | 3030 | 1471 | 816 | 7.60 |
| 4 | 5.3 | 3.13 | 20.53 | 3020 | 1473 | 691 | 7.80 |
| 8 | 12.2 | 7.93 | 19.17 | 3020 | 1523 | 612 | 7.95 |
| 12 | 18.8 | 13.96 | 18.21 | 3010 | 1524 | 606 | 8.18 |
| 16 | 21.5 | 15.70 | 17.92 | 2980 | 1530 | 596 | 8.15 |
| 20 | 23.0 | 15.92 | 16.92 | 2970 | 1540 | 589 | 8.11 |
| 24 | 23.8 | 16.66 | 16.72 | 2950 | 1540 | 585 | 8.19 |
| 28 | 25.3 | 16.68 | 16.72 | 2940 | 1563 | 575 | 8.29 |
| 33 | 26.5 | 16.90 | 16.71 | 2930 | 1567 | 570 | 8.42 |

**Table 5.** Results obtained for the experimental run corresponding to 60 °C and pH 7.0

| Time (days) | $Q_g$ (l) | $Q_{methane}$ (l) | VS (g/l) | O.N. (mg/l) | A.N. (mg/l) | $PO_4^{3-}$ (mg/l) | pH |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 25.41 | 3030 | 1471 | 816 | 7.00 |
| 4 | 2.0 | 1.20 | 23.14 | 2900 | 1475 | 611 | 7.37 |
| 8 | 8.8 | 5.60 | 14.44 | 2850 | 1479 | 569 | 7.35 |
| 12 | 14.9 | 8.36 | 13.98 | 2500 | 1504 | 444 | 7.38 |
| 16 | 18.7 | 9.22 | 13.15 | 1900 | 1572 | 389 | 7.70 |
| 20 | 21.8 | 14.10 | 12.97 | 1890 | 1610 | 374 | 8.09 |
| 24 | 26.8 | 16.81 | 12.45 | 1880 | 1854 | 351 | 8.21 |
| 28 | 29.1 | 17.04 | 12.01 | 1870 | 1915 | 323 | 8.23 |
| 33 | 29.4 | 19.26 | 11.79 | 1840 | 2039 | 279 | 8.08 |

**Table 3.** Results obtained for the experimental run corresponding to 35 °C and pH 7.0

| Time (days) | $Q_g$ (l) | $Q_{methane}$ (l) | VS (g/l) | O.N. (mg/l) | A.N. (mg/l) | $PO_4^{3-}$ (mg/l) | pH |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 25.41 | 3030 | 1471 | 816 | 7.00 |
| 4 | 3.8 | 2.10 | 22.26 | 2900 | 1496 | 799 | 7.51 |
| 8 | 5.6 | 3.61 | 20.61 | 2970 | 1523 | 691 | 7.80 |
| 12 | 17.2 | 9.63 | 18.87 | 2950 | 1594 | 675 | 7.98 |
| 16 | 23.2 | 16.01 | 16.48 | 2920 | 1775 | 583 | 8.01 |
| 20 | 24.1 | 16.85 | 16.01 | 2900 | 1846 | 575 | 7.97 |
| 24 | 26.5 | 17.96 | 15.07 | 2845 | 2034 | 550 | 7.93 |
| 28 | 27.6 | 18.31 | 13.23 | 2730 | 2088 | 483 | 8.11 |
| 33 | 27.7 | 18.45 | 13.20 | 2530 | 2165 | 462 | 8.21 |

**Table 4.** Results obtained for the experimental run corresponding to 60 °C and pH 7.6

| Time (days) | $Q_g$ (l) | $Q_{methane}$ (l) | VS (g/l) | O.N. (mg/l) | A.N. (mg/l) | $PO_4^{3-}$ (mg/l) | pH |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 25.41 | 3030 | 1471 | 816 | 7.60 |
| 4 | 2.5 | 0.20 | 25.40 | 2930 | 1494 | 804 | 7.39 |
| 8 | 3.7 | 1.04 | 24.83 | 2820 | 1510 | 739 | 7.61 |
| 12 | 5.5 | 2.37 | 23.13 | 2610 | 1625 | 709 | 7.85 |
| 16 | 9.8 | 6.08 | 21.61 | 2490 | 1835 | 669 | 8.18 |
| 20 | 14.2 | 9.80 | 20.84 | 2380 | 1870 | 654 | 8.24 |
| 24 | 14.7 | 9.83 | 19.41 | 2370 | 1900 | 630 | 8.17 |
| 28 | 15.9 | 10.17 | 18.40 | 2365 | 1919 | 622 | 8.26 |
| 33 | 18.1 | 12.31 | 18.37 | 1960 | 2319 | 613 | 8.35 |

# 3
# Results and discussion

The results obtained for the experimental run performed at initial pH 7.6 and mesophilic temperature are given in Table 2. These results show an exponential relationship between the methane production and digestion time. A high gas production was observed at the first 16 days and a reduction after this digestion time. Volatile solids diminished considerably during the first 16 days and furtherly a less variation was observed. Organic nitrogen removal was minimum, only 3% of the initial value was removed after 33 days of digestion time, and in consequence ammonia nitrogen increased slightly. Orthophosphate decreased significantly which could be related with the high removal of volatile solids, which reached an amount of 34%. Under

such conditions 30% of orthophosphate content was reduced. The pH of the process increased significantly during the digestion time rising from 7.60 at the beginning of the experiment to 8.42 at the end, which could have an influence on the poor organic nitrogen decomposition.

Table 3 summarizes the results obtained at mesophilic temperature and initial pH 7.00. Under such conditions methane gas production rate was maximum at day 16 of digestion decreasing furtherly. Total methane gas produced was higher at this run than at the corresponding run to pH 7.6. Also, the volatile solids removal was higher compared to that observed in the previous experiment. Organic nitrogen removal increased with respect to the value obtained at pH 7.6. In consequence, ammonia nitrogen concentration increased during the digestion time achieving a maximum value of 2165 mg/l but process inhibition was not observed, probably because of the pH increased to a lower value compared to that achieved in the first run. Orthophosphate removal was 43%, value higher than that obtained at pH 7.6 (30%).

Table 4 presents the results obtained at thermophilic temperature and initial pH 7.6. The results show that an increase of temperature enhanced the organic nitrogen removal until a value near to 35%. Ammonia nitrogen concentration and pH increased to about 2300 mg/l and 8.35, respectively. Probably the increase of temperature facilitated the organic nitrogen decomposition. Volatile solids removal and methane gas production were lower compared to the values found in the similar run at 35 °C. Maximum rate of methane production was appreciated at the day 20 and furtherly decreased, while the value of total methane produced decreased compared to the corresponding value at mesophilic temperature (Table 2).

Table 5 shows the result obtained at pH 7.00 and thermophilic temperature. As can be seen, volatile solids removals increased compared to those observed at mesophilic temperature. A higher methane gas production was appreciated in this run with a maximum value achieved at day 20. The overall organic nitrogen removal was higher and a similar behaviour was observed with respect to orthophosphate compared to the corresponding values observed at mesophilic temperature. In consequence, ammonia nitrogen concentration increased to about 2000 mg/l, which was less than the value obtained at mesophilic temperature. The final pH was also lower than that obtained at mesophilic temperature and similar initial

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pH, showing a lower accumulation of ammonia nitrogen, which could partially scape with the biogas due to the high temperature.

## 3.1
## Kinetics of methane production
In order to characterize each experiment kinetically and thus facilitate comparisons the following kinetic equation for methane production [20] was used:

$$G = G_m[1 - \exp(-K_0 t)] \quad , \tag{1}$$

where $G$ (l) is the volume of methane gas accumulated at a given time $t$ (days); $G_m$ (l) is the maximum volume accumulated at an infinite digestion time and is the product of the initial substrate concentration and the yield coefficient of methane; $K_0$ (day$^{-1}$) is an apparent kinetic constant that includes the biomass concentration ($K_0 = K X$). The fact that the biomass concentration in the digesters ($X$) remains virtually constant (5.3–5.5 g VSS/l) facilitates interpretation of the results. In fact, on comparing the apparent kinetic constants it is seen that all of them are multiplied by the same factor ($X$).

Equation (1) coincides with that proposed empirically by Roediger in Edeline [20] to relate the volume of gas in a batch anaerobic digestion process. According to this equation, methane production conforms to a first-order kinetic model [20–23]. As can be seen in Fig. 1, curves were obtained coinciding with the predictions of Eq. (1). Thus, $G$ was zero at $t = 0$, and the rate of methane production became zero at $t = \infty$. Also, the slopes of the curves decreased with increasing time.

On the other hand, taking Napierian logarithms in Eq. (1), and ordering the terms, the following is obtained:

$$\ln[G_m/(G_m - G)] = K_0 t \quad , \tag{2}$$

indicating that $\ln[G_m/(G_m - G)]$ versus $t$ should give a straight line of slope equal to $K_0$ with intercept zero. Figure 2 shows the experimental data for all the cases studied. The value of $G_m$ has been considered to be equal
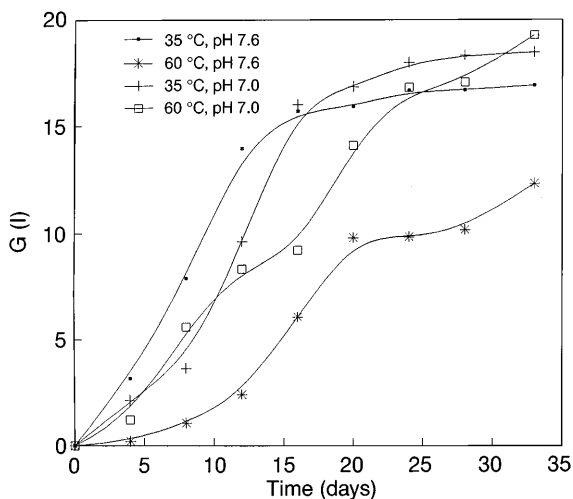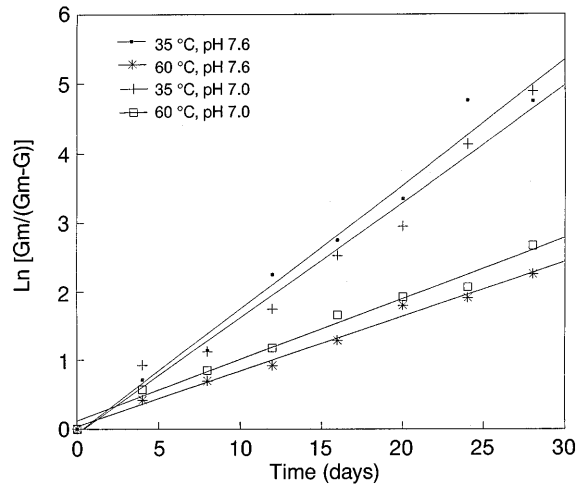


**Fig. 2.** Representation of the $\ln[G_m/(G_m - G)]$ values versus time (days) for the four cases studied

**Table 6.** $K_0$ values (days$^{-1}$) with 95% confidence limits for each experiment carried out

| Experimental conditions | $K_0$ (days$^{-1}$) |
|---|---|
| 35 °C, pH = 7.0 | 0.037 ± 0.003 |
| 35 °C, pH = 7.6 | 0.086 ± 0.004 |
| 60 °C, pH = 7.0 | 0.014 ± 0.009 |
| 60 °C, pH = 7.6 | 0.012 ± 0.007 |

to the volume of methane accumulated at the end of each experiment ($t = 33$ days). Representation of the experimental data as indicated, Eq. (2), gives straight lines with the intercept practically at zero. Thus, it is possible to fit the experimental data to the proposed model. Once it had been checked qualitatively that the experimental data of methane production conformed to the proposed model, the parameter $K_0$ was calculated analytically using a nonlinear regression program [24]. Table 6 lists the $K_0$ values with 95% confidence limits obtained for each case studied.

From the results it can be seen that the apparent kinetic constant of the process increased 2.3 times when the initial pH of the influent was increased from 7.0 to 7.6 at mesophilic temperature (35 °C). In contrast, the values were very similar at 60 °C temperature.

On the other hand, a decrease of the kinetics of methane production was observed when the temperature was increased from 35 °C to 60 °C, for the two initial pH values studied. Specifically, the apparent kinetic constant decreased 2.6 and 7.2 times when the operating temperature increased from 35 °C to 60 °C for the experiments carried out at initial pH of 7.0 and 7.6 respectively. This behaviour is due to the higher degradation rate of organic nitrogen to ammonia nitrogen observed at 60 °C, which is very toxic in the anaerobic digestion process. It is therefore common to find a wide range of values reported at which the anaerobic digestion process is deemed to have been inhibited by ammonia/amonium ion [25, 26]. It is, however, well accepted that toxicity effects may be detrimental to the anaerobic process performance in both homoge-



**Fig. 1.** Variation of the volume of methane accumulated, $G$(l), with time (days) for the experiments with different initial pH value and temperature

neous and fixed film systems. The anaerobic digestion of cow manure [27, 28] has been found to be very difficult at ammonia nitrogen concentration of 3 g/l; in these cases the authors attributed these difficulties to ammonia toxicity rather than high volatile solids loadings. The effect is likely to be more pronounced on the methanogenic population in the digester as these are reported to have the greatest sensitivity [29], *Methanobacterium formicum*, for example, was shown to be at least partially inhibited at an ammoniacal nitrogen concentration of 3.3 g/l. Not only do the variations in pH make it difficult to produce meaningfull figures relating to toxicity but there is also a growing weight of evidence which suggests that anaerobic consortium of bacteria can acclimatise to high ammonia concentrations [30–31]. A combination of both of these may explain the wide range of toxicity values reported and even account for high values such as those encountered by Parking and Speece [25] in completely mixed and biofilms reactors where ammoniacal nitrogen concentrations between 4 and 14 g/l have been observed.

The yield coefficient of methane, $Y_p$ (l $CH_4$ STP/g VS removed), was determined from the maximum methane volume produced (at the end of the process) and the initial and final VS values, which were known in each case (Tables 2–5). This yield coefficient decreased from 0.50 to 0.47 (initial pH 7.0) and from 0.65 to 0.58 l $CH_4$ STP/g VS removed (initial pH 7.6) for the experiments at 35 °C and 60 °C of temperature, respectively. This behaviour agrees with the major inhibition level observed at thermophilic temperature. In relation to the influence of the initial pH, $Y_p$ increased when the initial pH was increased at mesophilic temperature. For the thermophilic process the difference observed was quite less.

## 3.2
### Kinetics of organic nitrogen and phosphorus removal

Assuming that the overall anaerobic digestion process conforms to first-order kinetics [21, 22], the following equation was used [32–34] to obtain the specific rate constants ($K$) of organic nitrogen and phosphorus removal for the four cases studied:

$$S = S_0 \exp(-Kt) \; , \tag{3}$$

where $S_0$ and $S$ are the substrate concentrations (g COD/l) at the beginning and at a time $t$ of the digestion process, respectively. Table 7 shows the values of the specific rate constants for organic nitrogen and phosphorus removal.

**Table 7.** Values of the specific rate constants for organic nitrogen and phosphorus removal with 95% confidence limits for each case studied

| Experimental conditions | $K$ (days$^{-1}$) | |
|---|---|---|
| | Organic nitrogen removal | Phosphorus removal |
| 35 °C, pH = 7.0 | 0.005 ± 0.003 | 0.018 ± 0.003 |
| 35 °C, pH = 7.6 | 0.001 ± 0.002 | 0.005 ± 0.002 |
| 60 °C, pH = 7.0 | 0.018 ± 0.006 | 0.026 ± 0.007 |
| 60 °C, pH = 7.6 | 0.012 ± 0.008 | 0.009 ± 0.006 |

The value of the kinetic constant for organic nitrogen removal increased 3.6 and 12 times when the temperature was increased from 35 °C to 60 °C for the experiments carried out at initial pH values of 7.0 and 7.6, respectively. In relation to phosphorus removal, the values of the kinetic constants in the digesters at 60 °C are 44% and 80% higher than those obtained at 35 °C for the two initial pH values above-mentioned, respectively. With respect to the influence of the initial pH, a decrease of the kinetic constant of organic nitrogen and phosphorus removal was observed when the initial pH value increased from 7.0 to 7.6, being this variation greater at 35 °C of temperature.

In order to check the goodness of fit the experimental organic nitrogen and phosphorus concentrations were plotted against their theoretical counterparts (Figs. 3
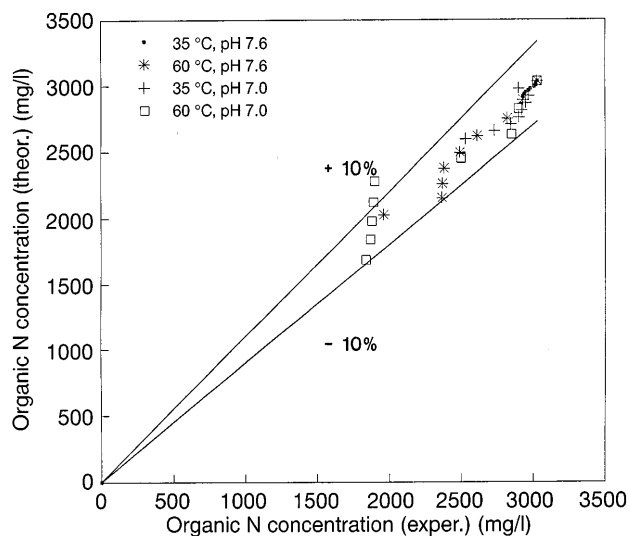
**Fig. 3.** Comparison between the experimental values of organic nitrogen concentration (mg/l) and those predicted by Eq. (3) for the four cases studied
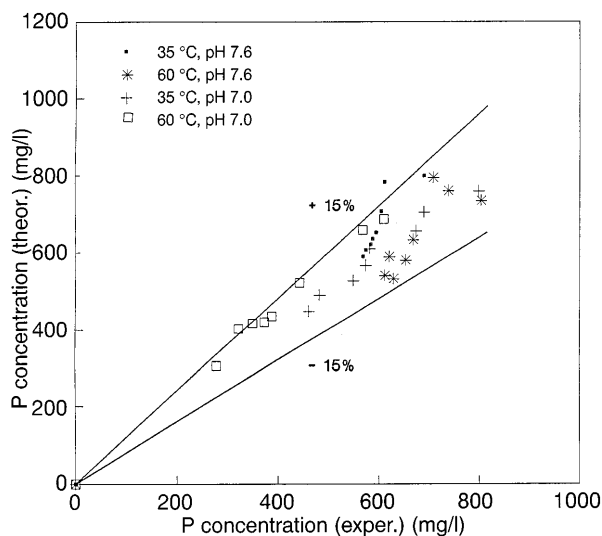


**Fig. 4.** Comparison between the experimental values of phosphorus concentration (mg/l) and those predicted by Eq. (3) for the four cases studied

and 4), which were calculated from the kinetic constants listed in Table 7. As can be seen, the experimental results were reproduced with errors equal to or less than 10% and 15% for organic nitrogen and phosphorus concentrations, respectively.

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