

## Anaerobic Treatment of Swine Wastewater in Semicontinuous Clayey Support Reactors

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Three micronized clayey supports in laboratory-scale tank reactors under mesophilic conditions were studied. From the results obtained in a previous work, the temperature of  $\theta = 25$  °C was chosen. The start up and performance of these bioreactors, operating on swine wastewater feed, were also studied. The anaerobic treatment in a semicontinuous regime was carried out.

Four stirred tank reactors were used, one of them containing suspended biomass for reference, while the rest contained various suspended micronized clay supports. The supports chosen were zeolite, esmectite, and saponite.

The higher removal efficiency (about  $\eta_{\text{COD}}$  70 % to 3.6 d HRT) was obtained when HRT increased in saponite and esmectite support reactors. Esmectite support showed the best anaerobic activity of microorganisms ( $\mu_{\text{max}}$  equal to  $0.576 \text{ d}^{-1}$ ). The reactor with zeolite support and the reference (non-support reactor) showed the worst yield in methane.

The methane production model proposed by Chen and Hashimoto to achieve an optimum purifying performance was carried out. It accurately predicted the performance of the process and may be used in the design of treatment units.

*Key words:*

Anaerobic digestion, semicontinuous process, clayey support, swine wastewater

### Introduction

Swine wastewater constitutes a grave environmental problem because of its high organic pollutant load and considerable volume. It affects soils as well as surface and ground waters, and is irritating because of its bad smell and proliferation of insects.<sup>1,2,3</sup> Large volumes of high-strength wastewaters are produced annually from industrial and agricultural operations. It is estimated that  $5.8 \cdot 10^7$  tons of animal manure are generated each year in the US<sup>4</sup> and almost  $1.2 \cdot 10^7$  tons of pig manure in the EU.<sup>5</sup> The swine wastewater must be treated to meet discharge regulations prior to being released into the environment<sup>6,7</sup> to avoid water contamination (nitrogen, phosphorous, copper, zinc, total organic carbon), and ambient problems (methane, ammonia and nitrous oxide emissions).<sup>8,9</sup>

Biological waste treatment methods are best for residues with an essentially highly organic contaminant load. Of the treatments of this kind of wastewater, anaerobic digestion has been shown to be the most appropriate for reducing the contaminating factor of these waste products while at the

same time producing a biogas with methane content between 60–80 %, which could eventually cover a large part of the farm energy needs.<sup>10</sup> Furthermore, the applications of anaerobic digestion process reduce carbon dioxide emissions and allow a coupled post-composting process.<sup>11</sup>

Among the reactors commonly used for this purpose are stirred tank reactors, where bacteria colonize particles of support materials, thereby increasing the surface available for bacterial growth.<sup>12</sup> Earlier studies<sup>13</sup> showed the influence of some supports used to immobilize the microorganisms in the anaerobic digestion process (PVC and clayey supports, mainly).

A previous paper<sup>14</sup> showed the best results obtained working at three different temperatures ( $\theta = 25, 35$  and  $47$  °C) and five reactors (a reference, and four micronized calcined supports). As a result of that previous work, depending on the percentage of chemical oxygen demand, reactors with saponite and esmectite clayey supports were recommended to work at  $25$  °C. At  $47$  °C a rapid increase occurred in the concentration of  $\text{NH}_4^+$  with a load decrease in methane production, but, the zeolite support reactor provided the lowest levels of

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$\text{NH}_4^+$  in the effluents because of its ionic exchange capacity. It is well known that when a failure in anaerobic digestion occurs caused by toxic substances or inhibitors, as  $\text{NH}_4^+$ , methanogens are the most sensitive members of the anaerobic bacterial consortium. That is why zeolite has been chosen.

In the present paper, the objective is the applicability of the anaerobic swine wastewater digestion and the subsequent establishment of optimum operating conditions for adequate purification performance. A semicontinuous experiment with four stirred tank reactors was carried out, at 25 °C, and the experimental results were compared with theoretical results predicted from a kinetic model of biological treatment fitted to high organic strength wastes. The model is the methane production model proposed by Chen and Hashimoto,<sup>15</sup> where, given that the reduction in the contaminant load of the effluent must be in proportion to methane production, the following expressions are obtained for the methane production model:

$$B/(B_0 - B) = (\mu_{\max}/k') \cdot \tau - 1/k' \quad (1)$$

$$\tau = 1/\mu_{\max} + k'/\mu_{\max} \cdot [B/(B_0 - B)] \quad (2)$$

where:

$B$  is the number of litres of methane produced at STP per gram of organic matter added to the reactor.

$B_0$  is the number of litres of methane produced at STP per gram of organic matter added to the reactor for an infinite retention time.

$\tau$  is the retention time, d.

$\mu_{\max}$  is the maximum specific growth rate of the microorganisms,  $\text{d}^{-1}$ .

$k'$  is a dimensionless kinetic parameter.

The parameter  $B_0$  is obtained by means of the equation:

$$B = B_0 [1 - k'/(\tau/\tau_{\min} - 1 + k')] \quad (3)$$

applied to the case where  $\tau/\tau_{\min} > 1 - k'$ , where  $\tau_{\min}$  is the minimum retention time (d):

$$\tau_{\min} = 1/\mu_{\max} \quad (4)$$

## Materials and methods

**Equipment** – Four stirred anaerobic reactors were used. The experimental set-up consisted of 1-litre active liquid volume magnetically stirred anaerobic reaction units, at constant temperature (Fig. 1). The temperature was maintained at  $\theta = 25$  °C (precision of  $\pm 1$  °C). The biogas generated was passed through a solution of sodium hydroxide (3 mol  $\text{L}^{-1}$ ) to retain carbon dioxide, and the vol-

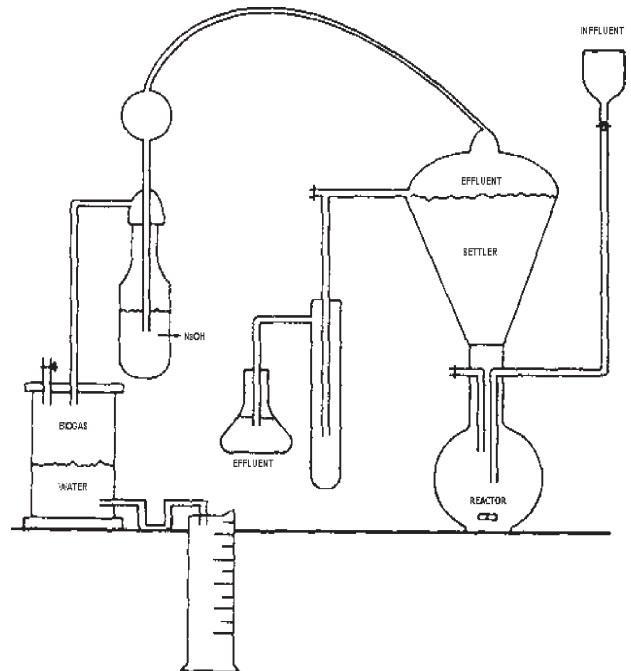


Fig. 1 – Experimental equipment used

ume of methane produced throughout this process was measured daily using 1 litre Mariotte reservoirs fitted to the reactors. The methane displaced a given volume of water from the reservoir, allowing ready determination of the biogas. The biomass was fluidized and agitated with the aid of a  $5 \cdot 15 \text{ cm}^2$  magnetic stirring system working at  $n = 160 \text{ min}^{-1}$ . The biological reactor included a settler assembly for reducing loss of biological solids and the solids acting as supports for the microorganisms.

The operation was in semicontinuous regime and the reactors were fed by means of a peristaltic pump; the effluent emerged from the reactor through a hydraulic seal including a 25 cm liquid column intended to prevent air from entering the reactor and biogas from leaving it.

**Inoculum and wastewater** – The wastewater used in this experiment was collected from “El Cerro” farm, Seville (Spain). Table 1 shows its characteristics.

Table 1 – Characteristics of the swine wastewater used in this experiment

|                            |                        |     |                        |
|----------------------------|------------------------|-----|------------------------|
| pH                         | 7.7                    | TS  | 20.0 g $\text{L}^{-1}$ |
| $\text{COD}_T$             | 25.0 g $\text{L}^{-1}$ | MS  | 6.6 g $\text{L}^{-1}$  |
| $\text{COD}_S$             | 7.8 g $\text{L}^{-1}$  | VS  | 13.4 g $\text{L}^{-1}$ |
| Alkaln ( $\text{CaCO}_3$ ) | 6.7 g $\text{L}^{-1}$  | TSS | 16.2 g $\text{L}^{-1}$ |
| VA (HAcO)                  | 0.5 g $\text{L}^{-1}$  | MSS | 3.1 g $\text{L}^{-1}$  |
| $\text{NH}_4^+\text{-N}$   | 1.6 g $\text{L}^{-1}$  | VSS | 13.1 g $\text{L}^{-1}$ |

The reactors were inoculated with biomass from an anaerobic pond of swine wastewater at the same farm. The mass concentration of biomass was  $\gamma_{VSS} = 9.0 \text{ g L}^{-1}$ . Characteristics of the inoculum, in  $\text{g L}^{-1}$ :  $\gamma_{TS} = 70.5$ ,  $\gamma_{MS} = 17.5$ ,  $\gamma_{VS} = 53.0$ ,  $\gamma_{TSS} = 62.7$ ,  $\gamma_{MSS} = 14.4$  and  $\gamma_{VSS} = 48.3$ , with a medium pH value of 7.6.

**Supports** – The materials used as supports for the bacteria were commercially available micronized esmectite, saponite, and zeolite ( $d = 2\text{--}5 \mu\text{m}$  diameter pore), supplied by Tolsa, S.A. (Madrid, Spain). Their characteristics are summarised in Table 2. These clayey supports were selected on account of their favourable kinetic behaviour from previous experiments.<sup>14</sup> A reference reactor without support was used.

Each reactor included  $15 \text{ g L}^{-1}$  of the above-mentioned supports.

Table 2 – Composition of the supports used<sup>a</sup>

|                                    | Saponite | Zeolite | Esmectite |
|------------------------------------|----------|---------|-----------|
| SiO <sub>2</sub>                   | 57.3     | 6.9     | 60.0      |
| Al <sub>2</sub> O <sub>3</sub>     | 4.4      | 11.9    | 17.3      |
| Fe <sub>2</sub> O <sub>3</sub>     | 2.0      | 2.1     | 5.3       |
| TiO <sub>2</sub>                   | 0.2      | –       | 0.2       |
| MgO                                | 25.4     | 1.2     | 6.0       |
| CaO                                | 0.6      | 2.8     | 0.5       |
| Na <sub>2</sub> O                  | 0.2      | 1.5     | 1.2       |
| K <sub>2</sub> O                   | 1.0      | 1.1     | 2.3       |
| calcination loss (1000 °C)         | 8.3      | 11.5    | 7.4       |
| moisture fraction (w/%)            | 9.0      | –       | 10.0      |
| bulk density (g mL <sup>-1</sup> ) | 0.84     | –       | 0.86      |

<sup>a</sup>Typical chemical analysis (% sample dried at 105 °C)

**Chemical analysis** – The following characterization quantities of the effluents were analyzed: pH, total chemical oxygen demand (COD<sub>T</sub>), soluble chemical oxygen demand (COD<sub>S</sub>), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N), volatile acids (VA), alkalinity, total solids (TS), mineral solids (MS), volatile solids (VS), total suspended solids (TSS), mineral suspended solids (MSS), and volatile suspended solids (VSS). These analytical determinations were made in accordance with the recommendations of the standard methods for the examination of water and wastewater.<sup>17</sup>

All experiments were conducted in duplicate.

**Experimental procedure** – In order to achieve optimum purification, a series of experiments were carried out at different retention times. The adapted period lasted 70 d, enough time to obtain an adequate attached biomass to the micronized particles.<sup>18</sup>

Experiments were carried out modifying the loading system, adding increasing hydraulic loads and decreasing the hydraulic retention time (HRT). The following HRTs were tested:  $t = 16.6, 8.3, 6.2, 5.0,$  and  $3.6 \text{ d}$ , giving volumetric organic loads ( $\Gamma$ ) ranging between  $1.5$  and  $7.0 \text{ g L}^{-1} \text{ d}^{-1}$  COD. Hydraulic retention time was maintained for three weeks to assure pseudostationary conditions. Once steady-state conditions were achieved at each feeding flow-rate methane production was measured each 24 hours, and the COD<sub>t</sub>, COD<sub>s</sub>, pH, alkalinity, VA, NH<sub>4</sub><sup>+</sup>-N, total, mineral and volatile solids, and total, mineral and volatile solids in suspension in the effluents were analyzed every 3 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 5 % in all cases.

## Results and discussion

### Analytical parameters: NH<sub>4</sub><sup>+</sup>-N, VA, alkalinity and solids

The concentration of NH<sub>4</sub><sup>+</sup> during the experience increased less than  $\gamma = 1 \text{ g L}^{-1}$  in the four reactors. The higher concentration was  $\gamma < 3 \text{ g L}^{-1}$ . This concentration is not recommended by some authors,<sup>19</sup> even though others demonstrate the acclimatization of the anaerobic microorganisms, specifically, to the high concentration of NH<sub>4</sub><sup>+</sup> in the swine wastewater.<sup>20</sup> It is known,<sup>21</sup> at the working temperature (25 °C) the NH<sub>3</sub>(g) (toxic N-molecule to microorganisms) dissolved is between 1.7–2.7 % in a pH range of 7.5 to 7.7, so its toxicity is minimal. The possibility of inhibition by high concentration of NH<sub>4</sub><sup>+</sup> is not considered.

As may be observed in Table 3, the VA/alkalinity ratio in the reference and zeolite reactors at high

Table 3 – VA/alkalinity ratio, for each  $\Gamma$  (g L<sup>-1</sup> d<sup>-1</sup>), HRT (d) and reactor

| $\Gamma$ | HRT  | Reference | Saponite | Zeolite | Esmectite |
|----------|------|-----------|----------|---------|-----------|
| 1.5      | 16.6 | 0.14      | 0.08     | 0.12    | 0.11      |
| 3.0      | 8.3  | 0.25      | 0.15     | 0.22    | 0.26      |
| 4.0      | 6.2  | 0.15      | 0.22     | 0.14    | 0.16      |
| 5.0      | 5.0  | 0.56      | 0.38     | 0.61    | 0.24      |
| 7.0      | 3.6  | 0.58      | 0.43     | 0.67    | 0.24      |

load exceeded 0.5, the value given in the literature<sup>22</sup> as the beginning of the microorganism inhibition process. This change coincides with a rapid decline in pH values in these two reactors (Fig. 2). Only the reactor with esmectite remained below 0.26, and this value was practically constant throughout the range studied, with a pH value of 7.7, higher than 7.5, optimum for methanogenic activity. This stability can be attributed to carbonate/bicarbonate buffering produced by the generation of CO<sub>2</sub> in the fermentation process, which is not completely removed from the reactor as biogas.

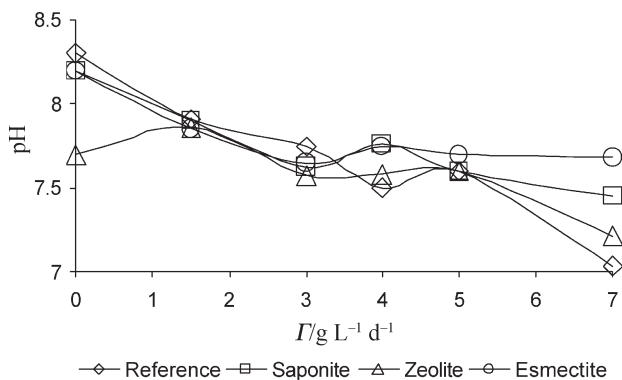


Fig. 2 – pH value vs  $\Gamma$  ( $g L^{-1} d^{-1}$ )

This phenomenon is understandable, since the methanogenic flora, the slowest to reproduce, have insufficient time to regenerate and the main activity is that of the acidogenic flora. Because of this, the degradation of organic matter goes no farther than intermediate products (volatile fatty acids, CO<sub>2</sub> and H<sub>2</sub>), stopping short of the final products (CH<sub>4</sub>).

Losses of MSS and VSS, and therefore of support and biomass with the effluent were smaller in the reactor with saponite than in the others, as was obtained in batch regime.<sup>14</sup> The reference reactor presented the highest losses of volatile suspended solids, almost an average value of 67 % with respect to the total volatile solids evacuated with the effluent, and so its capacity of retention is minimal, while reactors with zeolite, saponite and esmectite showed an average of 60 %, 50 % and 52 %, respectively. The clayey support used facilitated the retention of the biomass in the bioreactor as reported in the literature<sup>16,23</sup> with beet molasses and olive mill wastewater and in a previous paper<sup>14</sup> with swine wastewater in batch regime.

## Yields

In the reactors with saponite and esmectite, the production of CH<sub>4</sub> ( $Y_{CH_4}$ , mL L<sup>-1</sup> d<sup>-1</sup>) increased with volumetric load added. In contrast, under the same conditions, production decreased in the reference

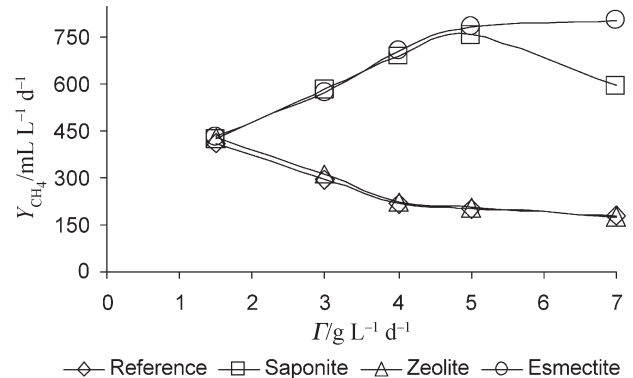


Fig. 3 – Production of methane ( $mL L^{-1} d^{-1}$ ) vs volumetric load added ( $g L^{-1} d^{-1}$ )

and zeolite reactors, with a very similar behaviour in the load range studied (Fig. 3).

Subsequently, the  $Y_{CH_4}$  parameter was calculated (eq. 5) which showed the fraction of organic substrate removed which had become methane. The figure of 350 corresponds to the volume of theoretical methane ( $V_{CH_4}$ , mL) obtained by g COD removed (STP), and  $\Gamma$  represents the organic load added.

$$Y_{CH_4} = V_{CH_4}/350 \Gamma \quad (5)$$

The values obtained upon applying eq. (1) are shown in Table 4. The volume of methane at 25 °C in STP conditions was used.

Table 4 –  $Y_{CH_4}$  (%) for each  $\Gamma$  ( $g L^{-1} d^{-1}$ ), HRT (d) and reactor

| $\Gamma$ | HRT  | Reference | Saponite | Zeolite | Esmectite |
|----------|------|-----------|----------|---------|-----------|
| 1.5      | 16.6 | 79        | 81       | 82      | 82        |
| 3.0      | 8.3  | 28        | 56       | 30      | 55        |
| 4.0      | 6.2  | 16        | 49       | 16      | 51        |
| 5.0      | 5.0  | 12        | 43       | 12      | 45        |
| 7.0      | 3.6  | 7         | 24       | 7       | 33        |

The fraction of substrate transformed into methane decreased with the load – up to 7 % in the reference and zeolite reactors, and up to 24 and 33 % in the saponite and esmectite reactors, respectively. The  $Y_{CH_4}$  in the last pair of reactors was higher working in semicontinuous regime than in batch regime<sup>14</sup> and that involves a high consumption of organic material that both reactors transform into methane.

## Purification efficiency

Knowing that the purification efficiency ( $\eta_{COD}$ , %) is a function of COD removed and COD initial

Table 5 – Purification efficiency ( $\eta_{COD}$ , %) with  $\Gamma$  ( $g L^{-1} d^{-1}$ ) and HRT (d) and reactor

| $\Gamma$ | HRT  | Reference | Saponite | Zeolite | Esmectite |
|----------|------|-----------|----------|---------|-----------|
| 1.5      | 16.6 | 95        | 93       | 94      | 93        |
| 3.0      | 8.3  | 79        | 86       | 80      | 87        |
| 4.0      | 6.2  | 68        | 81       | 67      | 81        |
| 5.0      | 5.0  | 56        | 73       | 57      | 76        |
| 7.0      | 3.6  | 53        | 64       | 52      | 73        |

rate, data from each reactor with HRT is shown in Table 5.

At an organic load of  $1.5 g L^{-1} d^{-1}$  (HRT = 16.6 d), the  $\eta_{COD}$  was 93–95 %. At high load, the efficiency of the reference-zeolite reactor pair decreased to 53 %, while that of the reactors with esmectite and saponite fell to 73 and 64 %, respectively, showing again the best behaviour.

Knowing the dependence between the degradable organic matter and the methane produced, a relationship was obtained between the purification efficiency parameter,  $\eta_{COD}$ , and  $Y_{CH_4}$  (both parameters showed a linear trend with HRT). The equations that correspond to the relationship  $Y_{CH_4}$  vs  $\eta_{COD}$ , for each reactor to 95 % of confidence, are shown below (eq. (6) to (9)):

**Reference reactor:**

$$Y_{CH_4} = 1.57 \cdot (\gamma_{So} - \gamma_S) / \gamma_{So} - 0.81 \quad (6)$$

**Reactor with saponite:**

$$Y_{CH_4} = 1.72 \cdot (\gamma_{So} - \gamma_S) / \gamma_{So} - 0.86 \quad (7)$$

**Reactor with zeolite:**

$$Y_{CH_4} = 1.62 \cdot (\gamma_{So} - \gamma_S) / \gamma_{So} - 0.84 \quad (8)$$

**Reactor with esmectite:**

$$Y_{CH_4} = 2.14 \cdot (\gamma_{So} - \gamma_S) / \gamma_{So} - 1.23 \quad (9)$$

The reactors with saponite and esmectite supports showed the best  $r$  coefficient values: 0.9686 and 0.9454, respectively. Knowing the  $\eta_{COD}$  for a concrete reactor, the  $Y_{CH_4}$  can be calculated. Expressing the whole expression for each parameter, the substrate mass concentration can be estimated from the value of methane produced, in the range of HRT from 16.6 to 3.6 d, when  $\gamma_{So}$  is equal to  $25 g L^{-1} COD$  (eq. 10).

$$\begin{aligned} (V_{CH_4} / 350) \div (\gamma_{So} / \tau) = \\ = slope \cdot (\gamma_{So} - \gamma_S) / \gamma_{So} - origin \end{aligned} \quad (10)$$

**Kinetics**

In order to compare experimental results with theoretical data predicted from a kinetic model, the methane production model proposed by Chen and Hashimoto was applied.

Fig. 4 plots the litres of methane produced at STP per gram of organic matter added to the reactors ( $B$ ) vs the inverse of the hydraulic retention time. This parameter behaves similarly for retention times between 8.3 and 3.6 d showing a linear regression curve where esmectite and saponite support reactors show the best data. Applying eq. (1), with  $B$  data vs HRT, the kinetic parameters of the methane production model can be obtained.

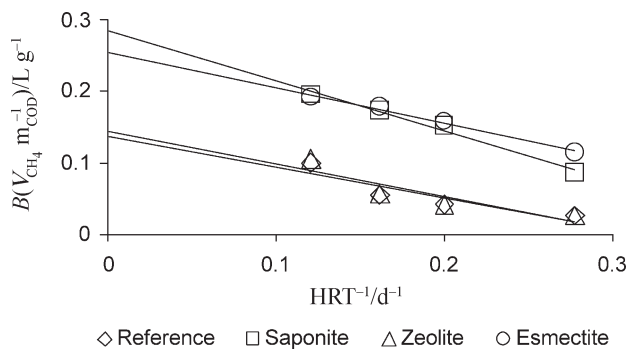


Fig. 4 – Litres of methane produced at STP per gram of organic matter added to reactor, expressed as COD vs inverse of HRT

Kinetic parameters in eq. (1) were derived as shown in Table 6 and Table 7:  $B_0$ , the number of litres of methane produced at STP per gram of organic matter added to the reactor for an infinite retention time;  $\mu_{max}$  is the maximum specific growth rate of the microorganisms ( $d^{-1}$ );  $k'$  is a dimension-

Table 6 –  $B/(B_0 - B)$  ratio for each HRT (d) and reactor

| HRT | Reference | Saponite | Zeolite | Esmectite |
|-----|-----------|----------|---------|-----------|
| 8.3 | 2.65      | 2.18     | 2.64    | 3.06      |
| 6.2 | 0.67      | 1.56     | 0.62    | 2.32      |
| 5.0 | 0.43      | 1.15     | 0.40    | 1.61      |
| 3.6 | 0.23      | 0.43     | 0.21    | 0.83      |

Table 7 – Kinetic parameters of the Chen-Hashimoto model

|              | Reference | Saponite | Zeolite | Esmectite |
|--------------|-----------|----------|---------|-----------|
| $\mu_{max}$  | 0.241     | 0.454    | 0.237   | 0.576     |
| $k'$         | 0.394     | 1.222    | 0.384   | 1.191     |
| $B_0$        | 0.136     | 0.284    | 0.145   | 0.254     |
| $\tau_{min}$ | 4.150     | 2.200    | 4.209   | 1.735     |

less kinetic parameter; and  $\tau_{\min}$  is the minimum retention time (d) ( $\tau_{\min} = 1/\mu_{\max}$ ).

The highest  $\tau_{\min}$  values in reference and with zeolite support reactors explains why from 5 d experimental retention time a marked instability is shown. In the same way, the highest value of  $\mu_{\max}$  may be seen with saponite and esmectite support, very similar to that found in the literature for cow-dung waste,<sup>7</sup> at 32.5 °C, and wine-vinasse<sup>15,16</sup> at 55 °C.

To confirm that the Chen and Hashimoto model fits with experimental methane data Fig. 5 shows  $B$  parameter from the methane-production model vs  $B$  calculated from experimental data obtained in the reactors with saponite and esmectite support, assuming that  $B_{\text{model}}$  is equal to zero when  $B_{\text{experimental}}$  is zero (eq. 11 and 12):

**Esmectite:**

$$B_{\text{model}} = 1.000 \cdot B_{\text{experimental}} \quad r = 0.9920 \quad (11)$$

**Saponite:**

$$B_{\text{model}} = 0.998 \cdot B_{\text{experimental}} \quad r = 0.9806 \quad (12)$$

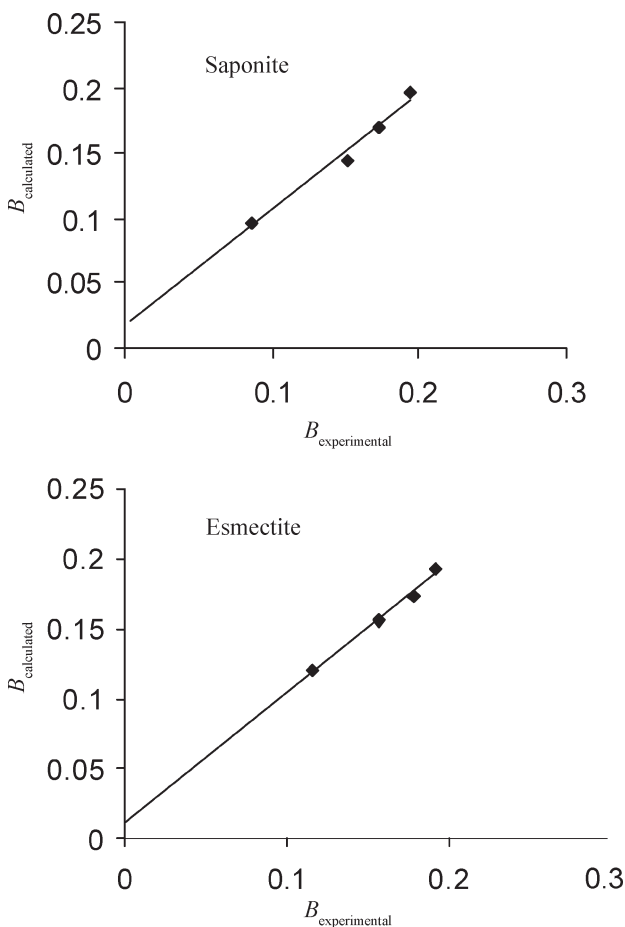


Fig. 5 –  $B$  estimated vs  $B$  experimental for the reactors with saponite and esmectite

## Conclusions

Esmectite support permits an anaerobic activity of microorganisms, similar to saponite support, due to its high apparent density, which avoids microbial losses. There are no important differences between these two supports because of their particular chemical composition. At the highest organic load added, the reactor with saponite support showed a loss of activity just due to an excessive store of solids inside the reactor.

Zeolite has an outstanding adsorbent capacity, but with increasing hydraulic load, the behaviour of this reactor becomes very similar to that of the reactor without support. Mainly, that is because of support losses with effluents.

From the VA/alkalinity values obtained, the maximum  $\Gamma$  working condition is 5.0 g L<sup>-1</sup> d<sup>-1</sup> (5.0 d HRT) with saponite support, and 7.0 g L<sup>-1</sup> d<sup>-1</sup> (3.6 d HRT) with esmectite support. That is a percentage removal of biodegradable COD of 73 % and 76 %, respectively.

The kinetic model proposed by Chen and Hashimoto proved suitable for forecasting the system's performance between 8.3 and 3.6 d of HRT. The maximum specific growth rate,  $\mu_{\max}$ , calculated by means of this model was 0.576 d<sup>-1</sup> with the best support used, esmectite, which corresponded to a minimum retention time of 1.735 d.

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## List of symbols

- $d$  – diameter,  $\mu\text{m}$
- $m$  – mass, g
- $t$  – time, d
- $V$  – volume, L
- $w$  – mass fraction, %
- $Y_{\text{CH}_4}$  – yield in methane,  $V_{\text{CH}_4} m_{\text{CODr}}^{-1}$ , mL g<sup>-1</sup>
- $\gamma$  – mass concentration, g L<sup>-1</sup>
- $\eta$  – removal efficiency of COD, %
- $\theta$  – temperature, °C
- $\mu$  – specific growth rate, d<sup>-1</sup>
- $\varphi$  – volume fraction of CH<sub>4</sub>, %
- $\eta_{\text{COD}}$  – removal efficiency, %
- $\mu_{\max}$  – maximum specific growth rate, d<sup>-1</sup>
- $B$  –  $V_{\text{CH}_4(\text{STP})} m_{\text{CODadded}}$ , L g<sup>-1</sup>

- $B_0$  –  $V_{\text{CH}_4(\text{STP})} m_{\text{CODadded}}$  for an infinite retention time,  
 $\text{L g}^{-1}$
- $\tau$  – retention time, d
- $k'$  – dimensionless kinetic parameter
- $\tau_{\text{min}}$  – minimum retention time, d
- $\Gamma$  – volumetric organic load,  $\text{g L}^{-1} \text{d}^{-1}$

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