Feed strategy study of a mechanically stirred anaerobic sequencing batch reactor (ASBR) equipped with a draft tube applied to whey treatment

Estudo da estratégia de alimentação de um reator mecanicamente agitado e operado em batelada sequencial equipado com draft tube aplicado ao tratamento de soro

Summary

An assessment was made regarding the effect of fill time on the stability and efficiency of an ASBR treatment of diluted whey. The reactor, with a work volume of 5 L, was mechanically stirred (75 rpm) and operated either in the batch or fed-batch mode. Assays were carried out at 30 ± 1 °C in 8 h cycles. The stirring system consisted of two impellers placed inside an internal axial-flow-promoting tube (draft tube). The impellers were a helix and an inclined turbine type, situated at the lower and upper part of the draft tube, respectively. The reactor was inoculated with 2 L of granular biomass and the alkalinity supplementation was maintained at a NaHCO₃/COD mass ratio of 50%. For the micro and macro nutrients the supplementation was added to the whey synthetic wastewater containing 400 mg.L⁻¹, in terms of COD. Feed strategies with fill times of 360, 180 and 10 min were assessed for the following conditions: a) influent organic matter concentration (CINF) of 4000 mg.L⁻¹ in terms of COD, and volume fed per cycle (Vfeed) of 1.0 L, and b) CINF = 8000 mg.L⁻¹ and Vfeed = 0.5 L. For both conditions, the organic load was maintained around 2400 mg.L⁻¹.d. The results showed that for condition a) the increase in fill time resulted in a decrease in organic matter removal efficiency and in an increase in total volatile acids concentration in the effluent. For condition b) the variation in fill time had no effect on organic matter removal efficiency. On the other hand, the total volatile acids in the effluent increased with increasing fill time. Thus condition a) for a fill time of 10 min presented the best results, with the lowest total volatile acids concentrations in the effluent (27 mgHAc.L⁻¹) and high organic matter removal efficiency for both filtered (98%) and unfiltered samples (94%).

Key words: Whey; ASBR; Anaerobic treatment.
Resumo

Este trabalho avaliou a influência do tempo de enchimento sobre a estabilidade e a eficiência de um reator anaeróbio operado em batelada sequencial (ASBR) no tratamento de soro de queijo diluído. O reator com 5 L de volume útil foi agitado mecanicamente (75 rpm) e operado em batelada simples ou alimentada. Os ensaios foram realizados a 30 ± 1 °C com ciclos de 8 h. O sistema de agitação foi composto por dois impelidores inseridos em um tubo interno (draft tube), dos tipos hélice e turbina inclinada, localizados nas partes inferior e superior do tubo interno, respectivamente. O reator foi inoculado com 2 L de biomassa granulada, havendo a suplementação de alcalinidade em 50% da razão mássica NaHCO$_3$. DQO$^{-1}$ e a suplementação de micro e macro nutrientes ao esgoto sintético contendo 400 mgDQO.L$^{-1}$. Avaliaram-se as estratégias de alimentação com tempos de enchimento de 360, 180 e 10 min para as seguintes condições: a) concentração de matéria orgânica afluente (CAF) igual a 4000 mgDQO.L$^{-1}$ e volume alimentado por ciclo (Valim) igual a 1,0 L; e b) CAF = 8000 mgDQO.L$^{-1}$ e Valim = 0,5 L. Em ambas as condições, a carga orgânica foi mantida em torno de 2400 mgDQO.L$^{-1}$.d. Os resultados mostraram que para a condição a) o aumento do tempo de enchimento do reator resultou na diminuição da eficiência de remoção da matéria orgânica e no aumento da concentração de ácidos voláteis totais presentes no efluente; e para a condição b) a variação do tempo de alimentação não teve efeito sobre a eficiência na remoção de matéria orgânica. Por outro lado, a concentração de ácidos voláteis totais presentes no efluente também aumentou com o aumento do tempo de enchimento do reator. Dessa forma, a condição a), para um tempo de enchimento de 10 min, foi a que apresentou os melhores resultados, com menor concentração de ácidos voláteis totais no efluente (27 mgHAc.L$^{-1}$) e elevada eficiência na remoção de matéria orgânica, tanto para amostras filtradas (98%) quanto para amostras não filtradas (94%).

Palavras-chave: Soro de queijo; Tratamento anaeróbio; ASBR.
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1 Introduction

The characteristics of wastewaters generated by dairy industries may vary widely depending on the type of product. In addition, the effluent usually consists of several streams with final organic matter concentrations of 2 to 5 gCOD.L\(^{-1}\). However, most cheese producers, especially small and medium-size ones, do not have sufficient resources and means to invest in technologies for the reuse of the cheese whey generated in the process, so they end up disposing their effluents in the waterways without any kind of treatment. This disposal generates great environmental problems since the polluting potential of cheese whey is very high.

The anaerobic treatment of whey is one of the most interesting alternatives to minimize this pollution problem. Different continuous anaerobic reactor configurations have been used in whey treatment and other dairy effluents (YAN et al., 1992; OMIIL et al., 2003; MALASPINA et al., 1995 and 1996; ERGÜDER et al., 2000; DEMIREL et al., 2005; GHALY et al., 2000), however in the cheese industry, whey is usually disposed of intermittently, making batch treatment units more attractive.

Feed strategy is one of the most important factors affecting the performance of anaerobic sequencing batch reactors (ASBR), the others including: stirring, initial ratio between substrate and biomass concentrations (F/M) and reactor configuration (ZAIAT et al., 2001). The influence of feed strategy is considerable since it is related to feed and total cycle time, influent COD concentration and volumetric organic load.

Reactor operation is considered to be batch when the fill time is negligible in relation to the total cycle length. On the other hand, fed-batch operation is characterized by a significant fill time in relation to the total cycle length. Fed-batch operation is employed for the following reasons: to prevent biomass inhibition by substrates or precursors, to minimize the effect of toxic products on the microorganisms, to reduce stability problems and to adjust the process to the operational conditions. Hence, when low concentrations of a certain substrate are desired, fed-batch operation is employed with either a constant or varying feed rate, according to the process interest.

According to Angenent and Dague (1995), an increase in feed time results in reduced availability of the substrate to the microorganism and causes less accumulation of volatile acids in the reactor. Bagley and Brodkorb (1999) suggested that longer feed times are beneficial to ASBR, especially when readily acidifiable substrates are used.

Rodrigues et al. (2003) employed a mechanically stirred ASBR containing granulated biomass to treat synthetic wastewater with an initial concentration of 500 mg.L\(^{-1}\) (in terms of COD). The reactor was operated at 30 ± 1°C and 50 rpm. The volume fed per cycle was 2 L and each cycle lasted 6 h, including 30 min for decanting. The feed strategies adopted were: 6 min (batch), 60, 120 and 240 min (batch followed by fed-batch) and 320 min (fed-batch). The authors concluded that different feed times did not affect system performance. Average substrate removal efficiencies were 78 and 84% for unfiltered and filtered samples, respectively. Moreover, the reactor showed stability, good solids retention as well as physical integrity of the granules under all adopted conditions.

A similar system was used by Ratusznei et al. (2003) containing immobilized biomass on polyurethane particles. The reactor was operated at 30 ± 1°C, 200 rpm and 180-min cycles. The feed strategies were: 3 min (batch) and 30, 60 and 180 min (fed-batch). During batch operation the system maintained stability and presented unfiltered sample organic matter removal efficiency of 86%. However, during fed-batch operation, the removal efficiency decreased. In addition, extra cellular polymer-like material was formed, jeopardizing contact between the substrate and the biomass due to low mass transfer between the medium and the microorganisms. This behaviour was also observed by Borges et al. (2004) when they submitted an ASBR to feed times that exceeded 50% of the total cycle length, which was 8 h.

Following the same research line to treat cheese whey, Damasceno et al. (2007) investigated the effect of three feed strategies (10, 120 and 240 min) on the performance of an ASBR. Organic loadings were 2, 4, 8 and 12 g.L\(^{-1}\).d and alkalinity was supplemented at a 50% NaHCO\(_3\).COD\(^{-1}\) mass ratio. A feed strategy of 120 min was shown to provide the best results regarding organic matter removal at loadings of 2 and 4 g.L\(^{-1}\).d, whereas a feed strategy of 240 min yielded better results at loadings of 8 and 12 g.L\(^{-1}\).d. Moreover, the maximum concentration of total volatile acids and minimum concentration of bicarbonate alkalinity did not change with changing feed strategies, but shifted along the cycle attaining a maximum (for acids) and minimum (for alkalinity) at the end of the feed period.

In virtue of the facts considered above and to continue these investigations, the main objective of this work was to assess the effect of feed strategy on the performance of a mechanically stirred ASBR equipped with a draft tube, applied in the treatment of whey. The fill time, influent concentration and treated volume per cycle were varied, whereas cycle length and applied organic load were maintained constant under the different conditions studied.

2 Material and methods

2.1 Experimental setup

The assays were carried out in a cylindrical acrylic reactor (diameter = height = 20 cm) that contained a mechanical stirring system for internal recirculation. The
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The work volume was 5.0 L. A central tube enclosing the impellers, known as a draft tube, provided for axial flow inside the reactor. The feed and discharge from the reactor were performed by means of diaphragm pumps connected to an automation system with timers for switching the pumps ‘on’ and ‘off’ and for activating the mechanical stirrer.

Stirring was implemented by two impellers: a helix impeller with three 6-cm blades and a turbine impeller with six inclined blades of the same size. The impellers were situated at the lower and at the upper part of the draft tube, respectively. The height and diameter of the draft tube were 10.5 and 7.5 cm, respectively. The temperature was controlled at 30 ± 1 °C, 8-h cycles were used and the stirring frequency was 75 rpm. The system is depicted in Figure 1 and the impellers of the stirring system are shown in Figure 2.

2.2 Wastewater

The influent wastewater consisted of reconstituted dehydrated cheese whey (3.0% water content) containing proteins (11.0%), carbohydrates (76.0%), lipids (1.0%) and ash (9.0%). In order to supplement the micro and macro nutrients and trace metals, the cheese whey was diluted in synthetic wastewater. This synthetic wastewater with a concentration of 400 mg.L⁻¹ in terms of COD, consisted of proteins (meat extract: 166.4 mg.L⁻¹), readily and unreadily degradable carbohydrates (sucrose: 28.0 mg.L⁻¹, amide: 91.2 mg.L⁻¹, cellulose: 27.2 mg.L⁻¹), lipids (soybean oil: 40.8 mg.L⁻¹) and salts (NaCl: 250 mg.L⁻¹, MgCl₂·6H₂O: 7.0 mg.L⁻¹, CaCl₂·2H₂O: 4.5 mg.L⁻¹). The alkalinity supplementation to the influent for buffering purposes was 0.5 g NaHCO₃.gCOD⁻¹.

It should be mentioned that the influent wastewater used in the experiments was always the same, i.e., a mixture of reconstituted dehydrated cheese whey and synthetic wastewater. Therefore the synthetic wastewater concentration in relation to the final influent concentration was always 400 mgCOD.L⁻¹ and the dehydrated cheese whey was added so as to attain the desired influent concentration. For instance, for an influent concentration of 4000 mgCOD.L⁻¹, the synthetic wastewater contribution was 400 mgCOD.L⁻¹ and that of the dehydrated cheese whey 3600 mgCOD.L⁻¹. Experimentally one gram COD corresponded to one gram dehydrated cheese whey.

2.3 Inoculum

The biomass used in all experiments came from an up-flow anaerobic sludge blanket reactor (UASB) treating poultry slaughterhouse wastewater. This inoculum presented total volatile solids (TVS) and total solids (TS) of 52 and 60 g.L⁻¹, respectively.

The reactor was inoculated with a granular anaerobic sludge containing 52 mg-TVS/g-sludge from a pilot UASB reactor treating domestic sewage. Two litres (2.081 g) of the sludge were added to the reactor, thus resulting in total solids and total volatile solids contents of 225 ± 25 g-TS and 195 ± 20 g-TVS, respectively, and biomass concentrations of 45 ± 5 g-TS/L (10 samples) and 39 ± 4 g-TVS/L (10 samples), assuming a volume of 5 L.

Figure 1. Scheme of the mechanically stirred anaerobic sequencing batch reactor [1 - Reactor (a = 20 cm; b = 16 cm; c = 8 cm; d = 20 cm); 2 - Medium vessel; 3 - Feed pump; 4 - Discharge pump; 5 - Draft Tube (e = 10.5 cm; f = 7.5 cm); 6 - Stirring system with turbine and helix impellers; 7 – Automation system; 8 – Discharge].

Figure 2. Details of the impellers used.
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2.4 Experimental protocol

The reactor was operated in the batch and fed-batch modes. The feed strategies implemented involved fill times of 180 and 360 min (fed-batch mode) and 10 min (batch mode), for the following conditions:

- **Condition a):** influent concentration \((C_{\text{INF}})\) of 4000 mg.L\(^{-1}\) (in terms of COD) and feed volume \((V_{\text{Feed}})\) of 1.0 L. Three assays were performed under this condition using fill times of 360 (strategy I), 180 (strategy II) and 10 min (strategy III);

- **Condition b):** influent concentration \((C_{\text{INF}})\) of 8000 mg.L\(^{-1}\) (in terms of COD) and feed volume \((V_{\text{Feed}})\) of 0.5 L. Three assays were performed under this condition using fill times of 360 (strategy I), 180 (strategy II) and 10 min (strategy III);

- **Condition c):** influent concentration \((C_{\text{INF}})\) of 2000 mg.L\(^{-1}\) (in terms of COD) and feed volume \((V_{\text{Feed}})\) of 2.0 L. Only one assay was performed using a fill time of 10 min (strategy III). This assay was used for subsequent comparison between the operational conditions in the batch mode (fill time of 10 min).

Thus the effect of different feed strategies was analyzed for the same organic load of 2.4 g.L\(^{-1}\).d and the same cycle length of 8 h. Despite the varying feed strategies, e.g., batch or fed-batch mode using fill times of 360 (strategy I), 180 (strategy II) and 10 min (strategy III), the decanting and discharging lasted 30 and 10 min, respectively, for all the conditions investigated. All the assays lasted 12 days or 36 cycles.

It should be pointed out that all the assays were carried out in 8-h cycles, i.e., three cycles per day. Thirty minutes were used for sedimentation, 10 min for discharge and the remaining 440 min for feeding and reacting. These values differed according to the feed step (strategy I, II or III) and the remaining time being for the reacting period. It should be mentioned that for the fed-batch operation (strategies I and II) significant reaction occurred during the feeding stage. In all assays the total volume of liquid medium in the reactor was 5 L. The volumes fed and discharged were according to the conditions a), b) or c).

It should be mentioned that the liquid level was always maintained above that of the draft tube to ensure perfect homogenization of the medium, otherwise the liquid external to the draft tube would not flow into the tube, and mixing would only be effective inside the tube.

2.5 Analysis

Monitoring was performed according to the Standard Methods for Examination of Water and Waste-

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- **Condition a):** \(C_{\text{INF}} = 4000\text{ mg.L}^{-1}\) and \(V_{\text{feed}} = 1.0\text{ L}\)

Table 1 shows the average values for the variables (influent and effluent) monitored during the assays with \(C_{\text{INF}} = 4000\text{ mg.L}^{-1}\) (in terms of COD) and \(V_{\text{feed}} = 1.0\text{ L}\). The reactor was shown to present high organic matter removal efficiency for each of the implemented feed strategies (fill times: strategy I – 360 min; strategy II – 180 min; and strategy III – 10 min). However, efficiency increased with decreasing feed time.

The values for total volatile acids (between 26.9 and 67.1 mgHAc.L\(^{-1}\) – Table 1) and bicarbonate alkalinity (between 1049.1 and 1790.7 mgCaCO\(_3\).L\(^{-1}\) – Table 1) in
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the effluent showed that the reactor maintained stability during the operation, as confirmed by the pH values (between 6.8 and 7.2 – Table 1). It can also be seen that despite the lower substrate concentrations in the reactor, an increase in fill time resulted in a slight increase in total volatile acids in the effluent, but this did not jeopardize process efficiency with respect to organic matter removal, which varied between 85.5 and 93.9% (Table 1) for unfiltered samples, and between 94.5 and 97.8% (Table 1) for filtered samples. Regarding the solids concentrations, no considerable biomass loss was observed, despite increases in the TS, TVS, TSS and VSS values as a result of the increase in fill time (Table 1).

Thus, increasing the feed period from 10 min (strategy III) to 180 min (strategy II) and then to 360 min (strategy I) increased the organic matter concentration in the influent at the end of the cycle for both unfiltered (from 274.3 to 686.8 mgCOD.L$^{-1}$ – Table 1) and filtered (from 97.3 to 259.5 mgCOD.L$^{-1}$) samples. However, this did not occur due to the increase in TVA (which varied from 26.9 to 67.1 mgHAc.L$^{-1}$), but may be due to the lower reaction rate with increasing feed time, since the average organic matter concentration decreased throughout the cycle, as can be seen in Figure 3.

Regarding the total volatile acids, an increased consumption was observed after feeding ended for strategies I ($t_{\text{feed}} = 360$ min) and II ($t_{\text{feed}} = 180$ min), i.e. after ending the fed-batch operation of a 480-min cycle (Figure 3). For the batch operation or strategy III ($t_{\text{feed}} = 10$ min), increased acidification was observed after the first two hours of the cycle and after the fourth hour, the system had already attained stability (Figure 3). No marked variation was seen in the bicarbonate alkalinity values for any of the three strategies investigated (Figure 3). The profiles for the intermediate volatile acid (IVA) concentrations were not conclusive, and no relationship could be drawn between the concentration of the acids formed and the increase in fill time. Hence, these results are not shown.

### 3.2 Condition b): $C_{\text{INF}} = 8000$ mg.L$^{-1}$ and $V_{\text{feed}} = 0.5$ L

Table 2 shows the average values for the variables (influent and effluent) monitored during the assays with $C_{\text{INF}} = 8000$ mg.L$^{-1}$ (in terms of COD) and $V_{\text{feed}} = 0.5$ L. The system was shown to present high organic matter removal efficiencies for both unfiltered and filtered samples and no significant difference were found between the three feed strategies adopted. The results obtained remained around 91% for unfiltered and 98% for filtered samples.

However, the organic matter concentrations at the end of the cycle under condition b) (Table 2) increased in relation to those under condition a) (Table 1). In addition, the organic matter concentrations increased when the feed time decreased. Considering the unfiltered samples and strategies III ($t_{\text{feed}} = 10$ min) and I ($t_{\text{feed}} = 360$ min), respectively, under condition a) the organic matter concentration varied from 274.3 to 686.8 mgCOD.L$^{-1}$, whereas for condition b) the variation was from 747.7 to 531.9 mgCOD.L$^{-1}$. This fact could be explained based on the relationship between the reaction rate and the organic

### Table 1. Average values for the variables monitored during condition a): $C_{\text{INF}} = 4000$ mg.L$^{-1}$, $V_{\text{feed}} = 1.0$ L and $t_{\text{feed}} = 360$, 180 and 10 min

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th>t$\text{feed} = 360$ min</th>
<th>t$\text{feed} = 180$ min</th>
<th>t$\text{feed} = 10$ min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{INF}}$ (mg.L$^{-1}$)</td>
<td>4577.8 ± 211.6$^{(2)}$</td>
<td>686.8 ± 107.7$^{(2)}$</td>
<td>514.2 ± 122.3$^{(2)}$</td>
<td>274.3 ± 24.4$^{(2)}$</td>
</tr>
<tr>
<td>$C_{\text{INF}}$ (mg.L$^{-1}$)</td>
<td>85.5 ± 2.3$^{(2)}$</td>
<td>88.5 ± 2.7$^{(2)}$</td>
<td>93.9 ± 0.5$^{(2)}$</td>
<td>97.3 ± 13.3$^{(2)}$</td>
</tr>
<tr>
<td>$C_{\text{INF}}$ (mg.L$^{-1}$)</td>
<td>259.5 ± 55.1$^{(2)}$</td>
<td>148.7 ± 21.2$^{(2)}$</td>
<td>96.7 ± 0.5$^{(2)}$</td>
<td>97.8 ± 0.3$^{(2)}$</td>
</tr>
<tr>
<td>$C_{\text{INF}}$ (mg.L$^{-1}$)</td>
<td>94.5 ± 1.2$^{(2)}$</td>
<td>96.7 ± 0.5$^{(2)}$</td>
<td>97.8 ± 0.3$^{(2)}$</td>
<td>26.9 ± 2.6$^{(2)}$</td>
</tr>
<tr>
<td>TVA (mgHAc.L$^{-1}$)</td>
<td>159.8 ± 35.5$^{(2)}$</td>
<td>67.1 ± 13.6$^{(2)}$</td>
<td>38.6 ± 13.5$^{(2)}$</td>
<td>26.9 ± 2.6$^{(2)}$</td>
</tr>
<tr>
<td>BA (mgCaCO$_3$.L$^{-1}$)</td>
<td>1358.9 ± 194.11$^{(2)}$</td>
<td>1790.7 ± 246.7$^{(2)}$</td>
<td>1049.1 ± 287.7$^{(2)}$</td>
<td>1353.3 ± 230.2$^{(2)}$</td>
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<tr>
<td>pH</td>
<td>8.0 ± 0.23$^{(9)}$</td>
<td>7.2 ± 0.16$^{(9)}$</td>
<td>6.8 ± 0.06$^{(9)}$</td>
<td>6.9 ± 0.06$^{(9)}$</td>
</tr>
<tr>
<td>TS (mg.L$^{-1}$)</td>
<td>5355 ± 116.2$^{(2)}$</td>
<td>2782 and 3328*</td>
<td>2002 and 456$^{(2)}$</td>
<td>1567 ± 247$^{(2)}$</td>
</tr>
<tr>
<td>TVS (mg.L$^{-1}$)</td>
<td>3791.0 ± 85.5$^{(3)}$</td>
<td>854 and 1218*</td>
<td>732 ± 148$^{(3)}$</td>
<td>533 ± 6$^{(3)}$</td>
</tr>
<tr>
<td>TSS (mg.L$^{-1}$)</td>
<td>143.0 ± 33.5$^{(9)}$</td>
<td>388 and 472*</td>
<td>347 ± 106$^{(9)}$</td>
<td>151 ± 58$^{(9)}$</td>
</tr>
<tr>
<td>VSS (mg.L$^{-1}$)</td>
<td>120.0 ± 23.2$^{(9)}$</td>
<td>340 and 372*</td>
<td>293 ± 86$^{(9)}$</td>
<td>134 ± 48$^{(9)}$</td>
</tr>
</tbody>
</table>

* Minimum and maximum values for the variables monitored; and numbers between brackets refer to the number of samples used for averaging.
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Table 2. Average values for the variables monitored during condition b): $C_{inf} = 8000$ mg.L$^{-1}$, $V_{feed} = 0.5$ L and $t_{feed} = 360, 180$ and 10 min.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th>Effluent</th>
<th>$t_{feed} = 360$ min</th>
<th>$t_{feed} = 180$ min</th>
<th>$t_{feed} = 10$ min</th>
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<tr>
<td>$C_{org}$ (mg.L$^{-1}$)</td>
<td>8848.5 ± 563.0(12)</td>
<td>531.9 ± 193.8(4)</td>
<td>784.3 ± 117.3(4)</td>
<td>747.7 ± 44.1(4)</td>
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<tr>
<td>$e_i$ (%)</td>
<td>-</td>
<td>91.8 ± 3.0(4)</td>
<td>90.9 ± 1.4(4)</td>
<td>91.9 ± 0.5(4)</td>
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</tr>
<tr>
<td>$C_{BA}$ (mg.L$^{-1}$)</td>
<td>-</td>
<td>122.7 ± 49.0(4)</td>
<td>176.4 ± 31.5(4)</td>
<td>172.0 ± 21.7(4)</td>
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<tr>
<td>$e_i$ (%)</td>
<td>-</td>
<td>98.1 ± 0.8(4)</td>
<td>98.0 ± 0.5(4)</td>
<td>98.1 ± 0.2(4)</td>
<td></td>
</tr>
<tr>
<td>TVA (mgHAc.L$^{-1}$)</td>
<td>262.2 ± 26.1(3)</td>
<td>127.7 ± 1.1(3)</td>
<td>73.5 ± 3.4(4)</td>
<td>70.1 ± 2.6(4)</td>
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<tr>
<td>BA (mgCaCO$_3$.L$^{-1}$)</td>
<td>2508.6 ± 488.1(3)</td>
<td>3043.6 ± 33.8(3)</td>
<td>2281.0 ± 240.8(4)</td>
<td>2184.0 ± 84.7(4)</td>
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<tr>
<td>pH</td>
<td>7.9 ± 0.4(9)</td>
<td>7.3 ± 0.11(5)</td>
<td>7.2 ± 0.08(4)</td>
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<tr>
<td>TS (mg.L$^{-1}$)</td>
<td>9802.3 ± 946.2(7)</td>
<td>3572 and 3896*</td>
<td>3746 and 3810*</td>
<td>3687 ± 180(5)</td>
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<tr>
<td>TVS (mg.L$^{-1}$)</td>
<td>6825.7 ± 585.3(7)</td>
<td>1022 and 300*</td>
<td>1138 and 1230*</td>
<td>1027 ± 87(3)</td>
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<td>VSS (mg.L$^{-1}$)</td>
<td>251.1 ± 41.8(7)</td>
<td>370 and 423*</td>
<td>467 and 510*</td>
<td>381 ± 115(3)</td>
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<tr>
<td>VSS (mg.L$^{-1}$)</td>
<td>194.9 ± 35.0(7)</td>
<td>277 and 323*</td>
<td>277 and 383*</td>
<td>324 ± 74(5)</td>
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</tbody>
</table>

* Minimum and maximum values for the variables monitored; and numbers between brackets refer to the number of samples used for averaging.

matter concentration. As the influent increases and the feed volume decreases (maintaining the same amount of substrate added) the dilution of the fed-batch becomes beneficial, but not sufficient to attain a concentration similar to that at the end of the cycle. Hence, feeding equal amounts of substrate (i.e., equal as applied to the organic load), but in different ways in terms of influent concentration and volume fed, affects the value of the organic matter concentration at the end of the cycle.

TVAs (between 70.1 and 127.7 mgHAc.L$^{-1}$ – Table 2) and BA (between 2184.0 and 3043.6 mgCaCO$_3$.L$^{-1}$ – Table 2) values showed that the reactor maintained stability for the three feed strategies investigated, as confirmed by the pH values in the effluent (between 7.1 and 7.3 – Table 2). However, an increase in fill time resulted in an increase in TVA concentration. For feed strategy I ($t_{feed} = 360$ min), this parameter was superior to 120 mgHAc.L$^{-1}$. Analogously with respect to the previous condition, this increase did not jeopardize reactor performance, as already indicated by the values for organic matter removal efficiency, BA and pH in the effluent (Table 2). It should be pointed out that fill time also alters the way in which the alkalinity is supplemented in the reactor, i.e. an increase in cycle length allows for a more gradual addition of alkalinity.

Regarding the solids concentrations, there was no considerable loss of biomass and the TS, TVS, TSS and VSS values were practically the same for the three feed strategies investigated (Table 2).

Conversion profiles during the cycle for the parameters $C_{org}$ (mg.L$^{-1}$), BA (mgCaCO$_3$.L$^{-1}$), TVA (mgHAc.L$^{-1}$) and IVA (mg.L$^{-1}$) were determined in the eighth cycle of each feed strategy. As shown in Figure 4, the feed strategy did not significantly interfere in reactor performance as regards organic matter removal, i.e., the fed-batch operations, or strategies I ($t_{feed} = 360$ min) and II ($t_{feed} = 180$ min) presented organic matter removal efficiency similar to that obtained in the batch operation, or strategy III ($t_{feed} = 180$ min). This same behaviour had already been observed during the system monitoring. As in the previous case (condition a – Figure 3), no organic matter concentration peaks were detected during the cycle.

With regard to total volatile acids (TVA), Figure 4 shows slight variations between the concentrations for the three strategies, especially between the third and sixth hours of the cycle, when the fed-batch operation was interrupted according to strategies I ($t_{feed} = 360$ min), and II ($t_{feed} = 180$ min), respectively (total cycle length of 480 min). However, during strategy I ($t_{feed} = 360$ min), an increase in acidification was observed at the end of the cycle, as already shown in Table 2. No marked variation was seen between the bicarbonate alkalinity values for strategies II ($t_{feed} = 180$ min) and III ($t_{feed} = 10$ min), respectively. When the reactor was operated in the fed-batch mode or strategy I ($t_{feed} = 360$ min), the bicarbonate alkalinity values oscillated during the cycle (Figure 4). Analogously with respect to the previous condition, the intermediate volatile acid (IVA) concentration profiles were not conclusive, and were not therefore, included in this paper.

3.3 Condition c): $C_{inf} = 2000$ mg.L$^{-1}$ and $V_{feed} = 2.0$ L

Table 3 shows the average values for the variables monitored (influent and effluent) during condition c) ($C_{inf} = 2000$ mg.L$^{-1}$, in terms of COD, with $V_{feed} = 2.0$ L). The reactor presented high organic matter removal efficiency, exceeding 90.0%, for both filtered and unfiltered samples. The TVA concentration (38.1 mgHAc.L$^{-1}$ – Table 3), BA (972.1 mgCaCO$_3$.L$^{-1}$ – Table 3) and pH (6.8 – Table 3) in the effluent showed that the reactor remained stable throughout the assay. As in the previous conditions no considerable biomass loss occurred during the cycle, as shown by the VSS values.
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Conversion profiles for the parameters $C_{FS}$ (mg COD L$^{-1}$), BA (mg CaCO$_3$ L$^{-1}$), TVA (mg HAc L$^{-1}$), IVA (mg L$^{-1}$) and biogas (mmol L$^{-1}$) were determined in the eighth cycle, as in the previous conditions. Figure 5 shows that the degradation of organic matter was very quick, and attained stability after 4 h. In this case, at the end of the cycle the organic matter concentrations in the filtered sample stabilized at values near 100 mg L$^{-1}$ in terms of COD.
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The TVA concentration presented a peak at the beginning of the cycle, which gradually decreased up to the fourth hour, when stability was attained. This means that the biomass metabolic activity was more intense at the beginning of the cycle, when the substrate concentration was higher. Moreover, in the same period, a slight change in the bicarbonate alkalinity concentration was observed, remaining practically constant throughout the cycle. The changes detected in the TVA and BA concentrations were confirmed by the pH profile (Figure 6), which showed a small drop at the beginning and slowly recovered during the cycle. The intermediate volatile acids with major effects on the process in terms of concentration, were acetic (between 17 and 65 mgHAc.L\(^{-1}\)) and propionic (between 7 and 108 mg.L\(^{-1}\)) acids.

The methane concentration in the reactor head space was 16 mmol.L\(^{-1}\), totaling 60% of the biogas generated. It should be mentioned that the methane concentration remained constant after four hours of the cycle.

In general, batch operations (strategies III – \(t_{\text{feed}} = 10\) min) presented better results; in addition to higher organic matter removal efficiencies, they presented the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{\text{TS}}) (mg.L(^{-1}))</td>
<td>2338.9 ± 162.9(^{(c)})</td>
<td>190.7 ± 34.2(^{(c)})</td>
</tr>
<tr>
<td>(\varepsilon_{\text{TS}}) (%)</td>
<td>91.8 ± 1.5(^{(c)})</td>
<td>85.7 ± 16.8(^{(c)})</td>
</tr>
<tr>
<td>(C_{\text{FS}}) (mg.L(^{-1}))</td>
<td>-</td>
<td>96.3 ± 0.7(^{(c)})</td>
</tr>
<tr>
<td>(\varepsilon_{\text{FS}}) (%)</td>
<td>-</td>
<td>81.6 and 111.1*</td>
</tr>
<tr>
<td>(\text{TVA (mgHAc.L}(^{-1}))</td>
<td>660.2 and 778.9*</td>
<td>972.1 and 152.2*</td>
</tr>
<tr>
<td>(\text{BA (mgCaCO}_3\text{.L}(^{-1}))</td>
<td>7.4 and 8.3*</td>
<td>6.8 ± 0.07(^{(c)})</td>
</tr>
<tr>
<td>(\text{TS (mg.L}(^{-1}))</td>
<td>3130 and 3236*</td>
<td>1638 and 2112*</td>
</tr>
<tr>
<td>(\text{TVS (mg.L}(^{-1}))</td>
<td>2088 and 2332*</td>
<td>466 and 794*</td>
</tr>
<tr>
<td>(\text{TSS (mg.L}(^{-1}))</td>
<td>80 and 104*</td>
<td>130 and 151*</td>
</tr>
<tr>
<td>(\text{VSS (mg.L}(^{-1}))</td>
<td>60 and 76*</td>
<td>106 and 122*</td>
</tr>
</tbody>
</table>

\(\*\) Minimum and maximum values for the variables monitored; and numbers between brackets refer to the number of samples used for averaging.

Figure 5. Organic matter concentration profiles for the filtered samples \(C_{\text{FS}}\), total volatile acids (TVA) and bicarbonate alkalinity (BA) during the cycle for condition c): \(C_{\text{INF}} = 2000\) mg.L\(^{-1}\), \(V_{\text{feed}} = 2.0\) L and feed strategy III \(t_{\text{feed}} = 10\) min.

Figure 6. pH profile during the cycle for condition c): \(C_{\text{INF}} = 2000\) mg.L\(^{-1}\), \(V_{\text{feed}} = 2.0\) L and feed strategy III \(t_{\text{feed}} = 10\) min.
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lowest TVA concentrations in the effluent. With regard to organic matter removal, the three conditions showed similar efficiencies, exceeding 90%. However, the lowest residual TVA values in the system were presented by condition a), i.e., $C_{INF}$ of 4000 mg·L$^{-1}$ and $V_{feed}$ of 1.0 L.

Regarding fed-batch operations, or strategies I ($t_{feed} = 360$ min) and II ($t_{feed} = 180$ min), for the same organic load of 2.4 g·L$^{-1}$·d, condition a) was once again shown to be the best application. This condition showed the best organic matter removal efficiency (exceeding 90.0%), in addition to the lowest effluent TVA concentration (26.9 mgHAc·L$^{-1}$ – Table 1).

Microbiological analyses detected no morphological difference between the inoculum sludge and the reactor sludge at the end of each experiment. Furthermore, microbiological analyses showed the existence of bacilli-like and vibria-like cells, as well Methanosarcina sp.-like and Methanoseta sp.-like archaeal cells. The last two were in population equilibrium.

4 Conclusion

For condition a), i.e., $C_{INF} = 4000$ mg·L$^{-1}$ and $V_{load} = 1.0$ L, an increase in fill time resulted in a decrease in organic matter removal efficiency. On the other hand, for operations with influent concentrations of 8000 mg·L$^{-1}$ and feed volumes of 0.5 L, a variation in fill time had no significant effect on carbonaceous organic matter removal efficiency. In both cases the fed-batch operations led to higher accumulation of TVA at the end of the cycle, without however jeopardizing system efficiency and stability.

In general, the batch operations ($t_{load} = 10$ min) were shown to give better results, since in addition to higher organic matter removal efficiencies, they also presented lower TVA concentrations in the effluent.

Comparing the three conditions investigated, condition a) with a fill time of 10 min, showed the best results, resulting in less total volatile acids in the effluent (26.9 mgHAc·L$^{-1}$) and high organic matter removal efficiency, for both filtered (97.8%) and unfiltered samples (93.9%).

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Notation

Symbols

- **BA** - Bicarbonate alkalinity (mgCaCO$_3$·L$^{-1}$).
- **$C_{INF}$** - Influent organic matter concentration (mg·L$^{-1}$).
- **$C_{FS}$** - Effluent organic matter concentration in filtered samples (mg·L$^{-1}$).
- **$C_{FS}$** - Effluent organic matter concentration in unfiltered samples (mg·L$^{-1}$).
- **IVA** - Intermediate volatile acids (mg·L$^{-1}$)
- **$t_{load}$** - Fill time of the reactor (min).
- **$t_{feed} / t_C$** - Ratio between reactor fill time and cycle length.
- **TS** - Total solids (mg·L$^{-1}$).
- **TSS** - Total suspended solids (mg·L$^{-1}$).
- **TVA** - Total volatile acids (mgHAc·L$^{-1}$).
- **TVS** - Total volatile solids (mg·L$^{-1}$).
- **$V_{feed}$** - Volume fed per cycle (L).
- **VSS** - Volatile suspended solids (mg·L$^{-1}$).

Greek letters

- **$\epsilon$** - Organic matter conversion efficiency in filtered samples (%).
- **$\epsilon_I$** - Organic matter conversion efficiency in unfiltered samples (%).

References


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