Operating feasibility of anaerobic whey treatment in a stirred sequencing batch reactor containing immobilized biomass

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Abstract The scope of this work was to evaluate the operating feasibility of anaerobic whey treatment in a stirred sequencing batch reactor (ASBR) containing biomass immobilized on inert support. Assays were performed using 8-hour cycles and agitation rate of 200 rpm at 30 ± 1°C, for treating cheese whey containing 500 to 4,000 mgCOD/L, which corresponded to a volumetric organic load (VOL) of 0.81 to 5.7 gCOD/L.d. Stability and high organic matter removal of about 96% were achieved at effluent concentration below 160 mgCOD/L for non filtered samples. Operating stability of the reactor was shown to be strongly dependent on the alkalinity supplementing strategy during the assay, especially during the startup period, where NaHCO₃ supplementation was approximately 20–30% of the chemical oxygen demand (mgNaHCO₃/mgCOD). After startup, alkalinity supplementation could be reduced down to 10% maintaining efficiency and stability. Moreover, proper homogenization of the system through mechanical agitation was also shown to be indispensable, especially with increasing organic load.

Keywords Anaerobic treatment; immobilized biomass; polyurethane foam; sequencing batch reactor; whey

Introduction Dairy whey is known to have very high organic strength with COD values ranging from as high as 60,000 to 80,000 mg/L (Yan et al., 1988; Podlech et al., 1991). By virtue of its characteristics some operating problems arise such as the difficulty of the biomass to granulate and the tendency to form a viscous polymeric material, likely of bacterial origin, severely reducing the settling capacity, which may cause loss of biomass. Hence, treating whey in anaerobic reactors requires care, due to the difficulties to maintain the stability of the operation (Malaspina et al., 1996; Yan et al., 1988).

Despite its very high biodegradability (about 90%), raw whey is a substrate lacking bicarbonate alkalinity and tends to acidify the environment very quickly, making it necessary to add bicarbonate, carbonate or some hydroxide supplement (Lo and Liao, 1986; Wildenauer and Winter, 1985).

Lack of alkalinity may be minimized by using a whey concentration lower than that in natura according to results from Yan et al. (1988) in UASB reactors. Kato et al. (1994) also studied this variable, however, for systems with concentrations of less than 1,000 mgCOD/L. Another alternative is to use liquid phase recycle, allowing alkalinity addition and dilution of the influent (Malaspina et al., 1996). Separation of the acidogenic phase from the methanogenic phase have also been investigated (Garcia et al., 1991; Germirli et al., 1993; Yilmazer and Yenigün, 1999; Martins et al., 2000). Process stability may also be affected by the presence and kind of support, as in the work of Patel et al. (1999) who used different types of inert support materials such as charcoal, gravel, brick pieces, pumice stone and PVC pieces for treating whey in upflow anaerobic reactors.
Table 1 lists the main conditions studied and the results obtained on bench and pilot scale of several reactor types, operating in one or two phases, with or without recycle of the liquid phase, used in the anaerobic treatment of raw or diluted whey and varying the volumetric organic load, hydraulic retention time, influent temperature and concentration.

In this way, it can be seen that despite the numerous investigations, many topics still need further investigation, especially regarding operation of the reactor, since a major part of the tests has been performed in continuous reactors (usually UASB). Use of batch reactors is not common. The aim of this work is to evaluate the operating feasibility of anaerobic whey treatment in a stirred sequencing batch reactor containing biomass immobilized on inert support.

### Material and methods

The reactor used in this work consisted of a cylindrical glass column with a diameter of 15 cm (total capacity of 2.0 L) containing 1.0 L medium (useful volume – VUseful). Mechanical stirring was provided by a magnetic stirrer with rpm control. The inert support for immobilizing the anaerobic biomass consisted of polyurethane foam cubes with sides of 0.5 cm and apparent density of 23 kg/m³, placed in a basket, as shown in Figure 1 (Ratusznei et al., 2000).

The inoculum used was obtained from a pilot-UASB reactor treating domestic wastewater with the following characteristics: total suspended solids of 56 g/L and suspended volatile solids of 38 g/L. For all the conditions investigated, a litre of inoculum was mixed with 30 g of polyurethane foam particles, resulting in an average value of total solids (ST) of 1,926 ± 215 mg-solids/g-foam and an average total volatile solids (SV) of 1,237 ± 190 mg-solids/g-foam, hence the average amount of volatile solids in the reactor was 37 ± 6 g.

The wastewater consisted of dehydrated reconstituted whey yielding concentrations of 500, 1,000, 2,000, 3,000 and 4,000 mg/L. The ratio between the concentration in COD and

### Table 1 Main operating characteristics of some processes of anaerobic dairy whey treatment

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Reactor (volume)</th>
<th>Influent VOL (gCOD/L.d)</th>
<th>T (°C)</th>
<th>ε (%)</th>
<th>HRT (d)</th>
<th>C I (gCOD/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>DSFFR (3.5 L)</td>
<td>CW_{DN}</td>
<td>3.1–3.8</td>
<td>35</td>
<td>85–87</td>
<td>4.3–3.5</td>
</tr>
<tr>
<td>[2]</td>
<td>UFFLR (3.9 L)</td>
<td>CW_{US}</td>
<td>14</td>
<td>35</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>[3]</td>
<td>UASB (17.5 L)</td>
<td>CW_{DN}</td>
<td>0.91–8.14</td>
<td>33</td>
<td>97–99</td>
<td>4.56–41.1</td>
</tr>
<tr>
<td>[4]</td>
<td>UASB(a) (1 L)</td>
<td>CW</td>
<td>30</td>
<td>35</td>
<td>99</td>
<td>0.45</td>
</tr>
<tr>
<td>[5]</td>
<td>HUASBR (8 L)</td>
<td>DW</td>
<td>5.53–17</td>
<td>35</td>
<td>87–75</td>
<td>6–8</td>
</tr>
<tr>
<td>[6]</td>
<td>UASB(b) (3 L)</td>
<td>CW</td>
<td>1–28.5</td>
<td>35</td>
<td>95–99</td>
<td>5–77</td>
</tr>
<tr>
<td>[7]</td>
<td>CSTR(a) (1.5/4.5 L)</td>
<td>CW</td>
<td>1–9</td>
<td>35</td>
<td>90–94</td>
<td>5.4–6.8</td>
</tr>
<tr>
<td>[8]</td>
<td>STS(a) (8 L)</td>
<td>CW</td>
<td>35</td>
<td>35</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>[9]</td>
<td>UFAF(m) (6 L)</td>
<td>CW</td>
<td>35</td>
<td>95</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>[10]</td>
<td>UFAF(m) (6 L)</td>
<td>CW</td>
<td>35</td>
<td>50</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

**Notation:** VOL – volumetric organic loading; T – temperature; ε – efficiency; HRT – hydraulic retention time; C I – influent concentration

**Reactor:** DSFFR – downflow stationary fixed-bed reactor; UFFLR – upflow fixed film loop reactor; UASB – upflow anaerobic sludge blanket; HUASBR – hybrid upflow anaerobic sludge blanket reactor; CSTR – continuous stirred tank reactor; UFAF – upflow anaerobic filter; STS – batch stirred tank reactor

**Superscript:** a – acidogenic phase; m – methanogenic phase; b – bench scale; p – pilot scale

**Influent:** CW_{DN} – diluted and neutralized cheese whey; CW_{US} – undiluted sour whey; CW – cheese whey

in mg/L was 1:1. Daily preparations of this influent were made to maintain its characteristics during the experiments. The reactor operated at 30 ± 1°C with agitation of 200 rpm and sequencing cycles of 8 hours, treating a volume of 0.5 L per cycle (V_{Daily Treated} = 1.5 L/d). The fill time, i.e. time to feed 0.5 L of influent was 3 min, reaction time 472 min and discharge time 3 min, completing an 8 h-cycle.

Tests were performed in three phases: (a) investigation of the effect of volumetric organic load on the efficiency and stability through assays with influent whey of 500, 1,000, 2,000, 3,000 and 4,000 mg/L; (b) investigation of the effect of agitation on the efficiency and mainly on the operating stability of the system through assays with whey concentration of 4,000 mg/L; and (c) investigation of the startup strategy of the reactor through assays with influent whey concentrations varying from 500 to 3,000 mg/L. In all assays performed, the amount of sodium bicarbonate added as alkalinity and, consequently, to buffer the medium, was varied in a way to obtain a minimum amount at which process stability and efficiency could be accomplished.

In phase (a) the system operated as follows (see Table 2): (a.1) with a whey concentration of 500 mg/L for 47 days (141 cycles), and addition of sodium bicarbonate from 200% to 10% in relation to the whey concentration; (a.2) with whey concentration of 1,000 mg/L for 23 days (69 cycles), and addition of sodium bicarbonate from 10% to 5% in relation to the whey concentration; (a.3) with whey concentration of 2,000 mg/L for 30 days (90 cycles), and addition of sodium bicarbonate from 10% to 5% in relation to the whey concentration; (a.4) with whey concentration of 3000 mg/L for 32 days (96 cycles), and addition of sodium bicarbonate from 20% to 10% in relation to the whey concentration; (a.5) with whey concentration of 4,000 mg/L for 30 days (90 cycles), and addition of sodium bicarbonate from 30% to 10% in relation to the whey concentration.

In phase (b) the system operated (see Table 3) with whey concentration of 4,000 mg/L for 48 days (144 cycles), and addition of sodium bicarbonate from 100% to 10% in relation to the whey concentration.

In phase (c) the system operated (see Table 3) with whey concentration varying from 500 to 3000 mg/L, in a total period of 35 days (105 cycles), and addition of sodium bicarbonate from 20% to 10%.

The substrate concentrations, in COD, for nonfiltered \( C_{ST} \) and filtered samples \( C_S \), total volatile acids (TVA), bicarbonate alkalinity (BA) and total and volatile solids were performed according to the procedure described in Standard Methods for Examination of Water and Wastewater (1995). The discharged volume and pH were also monitored in the influent and effluent for all conditions. The removal efficiencies, based on the nonfiltered

**Figure 1** Scheme of the anaerobic batch reactor [(a) 1 – reactor; 2 – feed pump; 3 – discharge pump; 4 – reservoir containing dairy whey; 5 – effluent; 6 – basket containing immobilized biomass; 7 – magnetic stirrer; 8 – gas outlet; 9 – stirring bar and (b) basket in details] (Ratusznei et al., 2000)

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(ε_T) and filtered samples (ε_S) of the effluent, always considered as nonfiltered samples of the influent (C_I), and volumetric organic load (VOL) were calculated through Eqs (1), (2) and (3), respectively.

\[ \varepsilon_T = \frac{C_I - C_{ST}}{C_I} \]  

(1) 

\[ \varepsilon_S = \frac{C_I - C_S}{C_I} \]  

(2) 

\[ \text{VOL} = \frac{V_{\text{Daily Treated}}}{V_{\text{Useful}}} \frac{C_I}{V_{\text{Useful}}} \]  

(3) 

**Results and discussion**

Initially, the operating feasibility of the anaerobic stirred sequencing batch reactor was evaluated at different volumetric organic whey strengths. These tests refer to phase (a), of which the influent feed strategies, regarding the amounts of sodium bicarbonate addition as a function of the test period, for the different concentrations implemented are listed in Table 2. The results obtained for the effluent are shown in Figures 2 to 6 for the tests with whey concentrations of 500, 1,000, 2,000, 3,000 and 4,000 mg/L, respectively.

The results obtained for a whey concentration of 500 mg/L, resulting in a volumetric organic load of 0.81 g COD/L.d, showed that despite the low concentration it was not possible for the system to operate without alkalinity supplementation. The strategy used to minimize the amount of sodium bicarbonate addition (see Table 2) allowed us to attain a level of 10% of this concentration in relation to the whey concentration from an initial relation of 100–200% with high process efficiency and stability, as shown in Figure 2. At this condition, the influent presented the following average values: pH = 8.0 ± 0.6, TSS = 61 ± 16 mg/L and VSS = 31 ± 12 mg/L, and the effluent: pH = 6.6 ± 0.6, TSS = 48 ± 18 mg/L and VSS = 21 ± 13 mg/L.

From these results, the supplementation strategy adopted for the assay with whey concentration of 1,000 mg/L and volumetric organic load of 1.6 mg COD/L.d was to start with the same amount as that added at the end of the previous assay. This seemed to be inadequate and was then duplicated (see Table 2). However, with stabilization of the system it was possible to reduce the previously implemented level and attain high efficiency and stable operation as shown in Figure 3. The influent, at this condition,

<table>
<thead>
<tr>
<th>Phase (a.1)</th>
<th>Phase (a.2)</th>
<th>Phase (a.3)</th>
<th>Phase (a.4)</th>
<th>Phase (a.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_I = 522 ± 9</td>
<td>C_I = 1,029 ± 14</td>
<td>C_I = 2,069 ± 52</td>
<td>C_I = 3,034 ± 71</td>
<td>C_I = 4,154 ± 91</td>
</tr>
<tr>
<td>VOL = 0.81 ± 0.6</td>
<td>VOL = 1.6 ± 0.1</td>
<td>VOL = 3.1 ± 0.2</td>
<td>VOL = 4.5 ± 0.2</td>
<td>VOL = 5.7 ± 0.5</td>
</tr>
<tr>
<td>t</td>
<td>B</td>
<td>BA</td>
<td>t</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>299</td>
<td>6</td>
<td>100</td>
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<tr>
<td>7</td>
<td>1,000</td>
<td>634</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>500</td>
<td>299</td>
<td>–</td>
<td>–</td>
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<tr>
<td>5</td>
<td>250</td>
<td>148</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>72</td>
<td>–</td>
<td>–</td>
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<tr>
<td>11</td>
<td>50</td>
<td>37</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

C_I = mgCOD/L; VOL = gCOD/L.d; t – day; B – mgNaHCO_3/L; BA – mgCaCO_3/L
presented the following average values: pH = 7.5 ± 0.5, TSS = 72 ± 13 mg/L and VSS = 43 ± 9 mg/L, and the effluent: pH = 6.2 ± 0.2, TSS = 50 ± 12 mg/L and VSS = 23 ± 5 mg/L.

At this point, it was observed that the amount of sodium bicarbonate initially added should be proportional to the organic load applied. Hence, the supplementation strategy used for assay with whey of 2,000 mg COD/L and volumetric organic load of 3.1 g COD/L.d was to add 10% sodium bicarbonate in relation to the whey concentration, reducing this value to 5% and 2.5% as soon as the system attained stability and high efficiency. However using a concentration of 2.5% did not seem to be safe and the concentration of 5% was again used, after a short period at 10%, with good performance of efficiency and stability as shown in Figure 4. This fact evidenced that restoring stability, after an unsuccessful attempt to reduce the amount of alkalinity addition, became more difficult at higher whey concentrations. At this condition, the influent presented the following average values: pH = 7.6 ± 0.4, TSS = 98 ± 23 mg/L and VSS = 79 ± 26 mg/L, and the effluent: pH = 6.4 ± 0.2, TSS = 46 ± 13 mg/L and VSS = 31 ± 9 mg/L.

Therefore, assays were performed with influent whey concentrations of 3,000 and 4,000 mg COD/L and volumetric organic load of 4.5 and 5.7 g COD/L.d, respectively. The alkalinity supplementation strategy consisted in utilizing 20% and 30% sodium bicarbonate in relation to the COD, respectively, and gradually reducing these values to an optimum of 10% in both cases, as shown in Table 2 and Figures 5 and 6. For the condition of 3,000 mg/L, the influent presented the following average values: pH = 7.8 ± 0.5, TSS = 176 ± 11 mg/L and VSS = 138 ± 9 mg/L, and the effluent: pH = 6.6 ± 0.2, TSS = 77 ± 11 mg/L and VSS = 44 ± 10 mg/L. For the condition of 4,000 mg/L, the influent presented the following average values: pH = 7.9 ± 0.7, TSS = 184 ± 30 mg/L and VSS = 159 ± 28 mg/L, and the effluent: pH = 6.8 ± 0.2, TSS = 67 ± 12 mg/L and VSS = 42 ± 9 mg/L.

It should be pointed out that in all assays formation of yellowish viscous material was detected, of which the coloration seemed to be slightly proportional to the influent concentration. However, this did not cause operational problems during the assays. Yet, before every change in conditions, the reactor was disassembled and the basket containing the bed of inoculated foam was washed in order to maintain the same initial conditions in all assays.

Phase (b) of the work was carried out by implementing disturbances in the agitation during the assay, using a whey concentration of 4,000 mg COD/L, of which the results are shown in Figure 7. After attaining stability, and maintaining a sodium bicarbonate concentration in the influent of 25% in relation to the whey concentration, agitation was interrupted at the 10th day of assaying for 1 day (or 3 cycles), which resulted in a significant drop in
performance regarding organic matter removal and a pronounced increase in volatile acids concentration. This behavior required increase in alkalinity supplementation in order for the system, on restoring agitation, to regain the previous levels of efficiency and stability, with a gradual reduction in alkalinity supplementation.

At the 23rd day of assaying, agitation was interrupted again, followed by a period of 8 hours without agitation (1 cycle) and 16 hours with agitation (2 cycles), for 3 consecutive
days, followed by 3 days with the usual agitation and then interrupted again for 2 days at periods of 8 hours without agitation (or 1 cycle) and 16 hours with agitation (or 2 cycles). As a result, the drop in performance in terms of organic matter removal and increase in volatile acids concentration was more significant yet, requiring increase in alkalinity supplementation for longer periods (see Table 3). However, this time, on restoring agitation, the system regained stability with gradual reduction of alkalinity supplementation, but without the same previous behavior, in relation to both substrate and volatile acids. In this condition, the influent presented the following average values $\text{pH} = 8.3 \pm 0.1$, $\text{TSS} = 201 \pm 25 \text{mg/L}$ and $\text{VSS} = 177 \pm 25 \text{mg/L}$, and the effluent: $\text{pH} = 7.1 \pm 0.3$, $\text{TSS} = 58 \pm 18 \text{mg/L}$ and $\text{VSS} = 44 \pm 21 \text{mg/L}$.

These results led to realization of phase (c), where it was possible to regain process efficiency and stability of the levels prior to phase (b), through a gradual increase in the volumetric organic load and adjustment of the amount of the bicarbonate added (see Table 3). In this way, operation of this system with high organic strength was shown to be possible by means of a startup strategy where there is gradual adaptation of the biomass, preventing significant accumulation of volatile acids and consequently minimizing alkalinity addition, as can be seen in Figure 8. In this condition, $\text{pH}$ of the influent was $8.1 \pm 0.2$ and of the effluent $6.6 \pm 0.2$.

![Figure 6](https://iwaponline.com/wst/article-pdf/48/6/179/423514/179.pdf)

**Figure 6** Results for whey concentration of 4,000 mg/L ($\Delta$ – $C_{\text{ST}}$; ■ – $C_{\text{SS}}$; △ – $\varepsilon_{T}$; □ – $\varepsilon_{S}$)

![Figure 7](https://iwaponline.com/wst/article-pdf/48/6/179/423514/179.pdf)

**Figure 7** Results for whey concentration of 4,000 mg/L – effect of stirring ($\Delta$ – $C_{\text{ST}}$; ■ – $C_{\text{SS}}$; △ – $\varepsilon_{T}$; □ – $\varepsilon_{S}$)

![Figure 8](https://iwaponline.com/wst/article-pdf/48/6/179/423514/179.pdf)

**Figure 8** Results for whey concentration of 500–3,000 mg/L – startup strategy ($\Delta$ – $C_{\text{ST}}$; ■ – $C_{\text{SS}}$; △ – $\varepsilon_{T}$; □ – $\varepsilon_{S}$)
Conclusions
Analysis of the result obtained in this investigation allowed us to arrive at the following conclusions: (a) it was possible to operate the sequencing batch reactor at stable conditions utilizing an 8-hour cycle and agitation of 200 rpm at 30°C, using cheese whey at concentrations from 500 to 4,000 mg/L, which correspond to volumetric organic loads of 0.81 to 5.7 g COD/L.d, values among those usually encountered in the literature; (b) process stability depends strongly on the alkalinity supplementation strategy as well as on mechanical agitation, requiring an initial supplementation of 20–30% in relation to the COD (mgNaHCO₃/mgCOD), being possible a reduction down to 10% and still maintaining high efficiency and stability; (c) The system attained high organic matter removal efficiency of about 96% with effluent concentration inferior to 160 mgCOD/L for the nonfiltered samples, considering all dairy whey concentrations studied.

In this way, the experiments performed allowed us to obtain interesting results regarding application of the anaerobic dairy whey treatment in a stirred sequencing batch reactor containing biomass immobilized on polyurethane foam.

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