Performance of an ECSB reactor for high-rate anaerobic treatment of cheese industry wastewater: effect of pre-acidiﬁcation on process efﬁciency and calcium precipitation
Vasileios Diamantis and Alexander Aivasidis

ABSTRACT
An external circulation sludge bed (ECSB) reactor was studied at full-scale (1,000 m³) during anaerobic treatment of cheese and other dairy products industry wastewater (CWW). The latter was characterized by a high calcium content, therefore the study focused on the potential negative impact that calcium may have in the long-term. The degree of CWW acidification (25 and 40%) on ECSB reactor performance was evaluated over a wide range of organic loading rates from 5 up to 18 kg m⁻³ d⁻¹, while process efﬁciency and calcium precipitation were examined in detail. Independently of the operating conditions, the volatile suspended solids content of the anaerobic granular sludge, as well as its calcium content, remained stable along the ECSB reactor operation, indicating that there was no calcium build up in the biomass. The results of this study demonstrate that the ECSB design seems to be particularly suitable to treat calcium-rich wastewater that is probably due to the fact that in this system CaCO₃ precipitates in the bulk liquid of the external circulation tank and not the biomass present in the main reactor, and that the CaCO₃ crystals are washed-out from it due to the high upflow velocity applied to the system (5 m h⁻¹).

Key words | acidification, anaerobic digestion, calcium precipitation, cheese whey, separated phase

INTRODUCTION
Organically polluted wastewaters are of major interest for biogas production since more than 4,000 full-scale anaerobic treatment systems are currently in operation worldwide (van Lier et al. 2013). Cheese whey, a byproduct from the dairy industry, is classiﬁed among the most studied substrates for methane generation. However, data from the operation of full-scale facilities are scarce (Demirel et al. 2005; Chatzipaschali & Stamatis 2012). Anaerobic digestion of cheese whey often results in process instabilities, mainly due to (a) the high concentration of readily degradable chemical oxygen demand (COD) (lactose), (b) low buffering capacity, (c) high salinity, (d) high calcium content and (e) the presence of proteins and lipids that entail low biodegradability (e.g. Vidal et al. 2000; Demirel et al. 2005; Chatzipaschali & Stamatis 2012). Under these conditions, high-rate anaerobic treatment systems may encounter severe propionic acid accumulation and increased consumption of NaOH for pH neutralization (Diamantis et al. 2014).

A parameter frequently underestimated in the anaerobic digestion of cheese whey, is the concentration of calcium, which may vary from 0.6 up to 1.5 g L⁻¹ (Danalewich et al. 1998; Carvalho et al. 2015). Calcium contains a necessary macronutrient for the anaerobic bacteria and it is of major importance for anaerobic sludge granulation (van Lier et al. 2015). It is, however, responsible for Ca²⁺ precipitation and cementation of reactor pipes, digester components and the anaerobic sludge itself, with negative consequences such as costly equipment maintenance and even bioreactor failure (see Figure S1 in Supplementary Material, available with the online version of this paper). Similar problems have been reported in different anaerobic treatment systems, treating calcium rich wastewaters, such as continuously stirred tank reactors (CSTR) (Marti et al. 2008), upﬂow anaerobic sludge bed (UASB) (van Langerak et al. 1998; 2000) and anaerobic membrane reactors (AnMBR) (You et al. 2006). The latter may encounter,
besides biomass cementation, severe flux decline due to membrane scaling (You et al. 2006) enabling the operation at extremely low permeate flux (\( \sim 2 \text{ L m}^{-2} \text{ h}^{-1} \)) (Al-Malack & Aldana 2006). Van Langerak et al. (1998, 2000) demonstrated that cementation of a UASB sludge bed occur within 180 d of reactor operation using a synthetic pre-acidiﬁed wastewater. Finally, CSTR digesters, designed with long hydraulic retention time, may precipitate calcium phosphate in the form of hydroxyapatite, which is thermodynamically the most stable form (Montastruc et al. 2003; Marti et al. 2008).

Cheese whey pre-acidiﬁcation consists of a pre-treatment process capable of improving the proceeding methanization step. It was demonstrated recently that a high degree of wastewater pre-acidiﬁcation resulted in stable UASB performance at high organic loading rate (up to 20 kg m\(^{-3}\) d\(^{-1}\)), with low volatile fatty acid (VFA) accumulation and negligible NaOH consumption (Diamantis et al. 2014). Besides, the acidification of carbohydrates at low pH conditions (<5), results in lactic acid, ethanol, acetic and butyric acid fermentation (Diamantis et al. 2014). Indeed, the fermentation to propionic acid is avoided, which is the rate limiting step of the overall methanization process. On the other hand, a high degree of wastewater acidification resulted in severe calcium precipitation as demonstrated by van Langerak et al. (1998). Therefore, a balance between acidification, enabling stable bioreactor performance, and limited calcium precipitation is necessary.

In this study, an external circulation sludge bed (ECSB) reactor was operated for 2 years under field conditions using cheese industry wastewater (a mixture of cheese whey and wastewater) over a wide range of organic loading rates (OLR) (up to 18 kg m\(^{-3}\) d\(^{-1}\)). The bioreactor was set in operation under a low or high degree of wastewater pre-acidiﬁcation to examine the effect on process performance and calcium precipitation. Bioreactor efﬁciency was evaluated based on COD removal, VFA accumulation, biogas production rate and methane yield. To our knowledge, this is the first study reporting long-term operation of a full-scale anaerobic facility treating cheese industry wastewater.

**MATERIALS AND METHODS**

**Wastewater origin and pre-treatment**

Wastewater that originated from equipment and ﬂoor washing (TYRAS S.A., Greece) was received after homogenization (870 m\(^3\) buffer tank) and separation of suspended solids using a dissolved air ﬂoatation process. Cheese whey produced in the same factory was stored in 5 × 100 m\(^3\) stainless steel tanks before use.

**Bioreactor design and operation**

An ECSB reactor, designed and built by the Dutch company HydroThane, having a 1,000 m\(^3\) working volume, and connected with a 60 m\(^3\) external circulation column reactor – so called Neutralization Tank – was used for the study (Figure 1). The bioreactor temperature was maintained at 29 ± 1°C. The anaerobic efﬂuent was continuously recirculated at ﬂowrate up to 300 m\(^3\) h\(^{-1}\), through the external circulation column reactor, where the generated biogas was also released (see Figure 1). pH regulation was performed inside the external circulation column reactor, using NaOH (45% w/w), and the former was maintained equal to 6.76 (±0.12). The upﬂow velocity of the ECSB reactor was 5 m h\(^{-1}\).

The ECSB reactor inﬂuent consisted a mixture of cheese whey and wastewater (so called cheese industry wastewater) at a ratio between 1:10 and 1:20, similar to previous studies (Liu et al. 2011). They were subsequently mixed into a 800 m\(^3\) working volume buffer tank before feeding the ECSB reactor. The inﬂuent wastewater ﬂowrate was equal to...
2,000 ± 230 m³ d⁻¹ corresponding to a hydraulic retention time of 12 ± 1.3 h inside the ECSB.

The study consisted of two periods: the first period (days 0–200) was conducted under a low and the second (days 200–800) under a high degree of wastewater pre-acidiﬁcation (26 ± 4 and 41 ± 4% VFA/COD soluble, respectively). The degree of wastewater pre-acidiﬁcation was regulated by varying the hydraulic retention time of cheese whey inside the storage tanks. During bioreactor operation, trace elements (iron, nickel, cobalt and molybdenum) were supplemented using a customized solution (Vitcomplete, HydroThane). Process efﬁciency was evaluated considering COD removal, biogas production rate, methane yield, COD and VFA accumulation, and the degree of Ca²⁺ precipitation. By the end of each experimental period, the anaerobic granular sludge was characterized for trace metal concentrations.

Anaerobic sludge activity

Anaerobic granular sludge samples were obtained by the end of each experimental period and assessed for methanogenic activity using 2 L working volume batch anaerobic reactors. The reactors were equipped with a magnetic stirrer operating at 200 rpm (Labino, model L-73), a thermal bath with hot water recirculation (LAUDA) and pH measurement and control (Endress Hauser). During the trials the pH was maintained at 7.22 (±0.40) and the temperature at 36 ± 1.5 °C. The sludge samples were fed with 6.6 g COD consisting 75% acetic and 25% ethanol. The experiments were conducted in duplicate and the biogas production from each bioreactor was monitored with an inverse water column, filled with alkaline water to remove CO₂. From the maximum methane production rate, the speciﬁc methanogenic activity (SMA) of the test biomass was calculated as g COD·CH₄ g⁻¹ VSS d⁻¹.

Analytical methods

During reactor operation, the inﬂuent ﬂowrate was measured with an electromagnetic ﬂow meter (Pro Mag, Endress-Hauser). The ﬂowrate of biogas was recorded using an ultrasound biogas meter (Prosonic, Endress-Hauser). The biogas methane content was monitored with an infrared biogas analyzer (BINOS). Samples were obtained daily from the buffer tank (reactor inﬂuent) and the reactor effluent for chemical analysis. They were characterized for COD total and soluble (TCOD and SCOD), pH, orthophosphates (PO₃⁻-P), total phosphorus, ammonia (NH₄⁻-N), total Kjeldahl nitrogen (TKN), calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺) and sodium (Na⁺) according to Standard Methods (APHA 1998). The determination of soluble COD, PO₃⁻-P, NH₄⁻-N and Ca²⁺ was performed after centrifugation (4,000 rpm for 5 min) and sample ﬁltration with 0.45 μm microﬁlters. VFA (acetic, propionic, butyric, iso-butyric and valeric acid) concentrations were measured through gas chromatography (Perkin Elmer Auto System XL), according to Diamantis et al. (2006). Anaerobic granular sludge suspended solids (TSS), volatile fraction of TSS and heavy metals concentrations were determined according to Standard Methods (APHA 1998). For this purpose, dry granular sludge samples were acid digested using HNO₃–HCl for 2 h. The trace element concentrations were determined in ﬁltered samples (pore size 0.20 μm) by using ICPMS (THERMO ICAP-QC ICP-MS).

RESULTS AND DISCUSSION

Wastewater characteristics

The cheese and dairy industry wastewater composition was affected by the daily quantity of cheese, yogurt and milk whey and (relatively low strength) wastewater generated at the industrial facility (see Supplementary Material, available with the online version of this paper). The CWW was acidic (pH = 4.83), having a TCOD concentration between 3,000 and 8,000 mg L⁻¹, on average 5,410 ± 1,350 mg L⁻¹. The concentration of SCOD was 4,160 ± 1,050 mg L⁻¹ consisting 77 ± 4% of the TCOD. The VFA were mainly acetic (40% of VFA as SCOD) and propionic acid (35%), followed by butyric (15%) and valeric (10%) acids.

Cheese whey was characterized by a high calcium and phosphorus content (790 and 560 mg L⁻¹, respectively) and this was subsequently decreased after dilution with low strength wastewater (Table 1). Similar calcium and TP concentrations were reported for cheese whey in previous studies (e.g. El-Mamouni et al. 1995; Malaspina et al. 1996; Ghaly et al. 2000). By the applied dilution, the CWW calcium content decreased to 136 ± 20 mg L⁻¹, corresponding to a COD:Ca ratio of 40:1. Indeed, this range was considered optimum for high-rate anaerobic wastewater treatment (Liu et al. 2011). Similarly, the cheese whey Na⁺ content displayed a considerable decrease from 3,300 to 470 mg Na⁺ L⁻¹. The COD:N:P ratio of CWW was 100:2.5:1.5, which is considered adequate for anaerobic digestion (Aivasidis & Diamantis 2005).
**Table 1** | Physicochemical properties of typical cheese whey, wastewater, and cheese industry wastewater samples examined during the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cheese whey</th>
<th>Wastewater</th>
<th>Cheese industry wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (-)</td>
<td>3.86</td>
<td>4.97</td>
<td>4.83</td>
</tr>
<tr>
<td>COD total (mg L⁻¹)</td>
<td>53,850</td>
<td>2,500</td>
<td>5,410</td>
</tr>
<tr>
<td>COD soluble (mg L⁻¹)</td>
<td>51,480</td>
<td>1,870</td>
<td>4,160</td>
</tr>
<tr>
<td>VFA (mg L⁻¹ as acetic)</td>
<td>970</td>
<td>280</td>
<td>2,110</td>
</tr>
<tr>
<td>Total P (mg L⁻¹)</td>
<td>530</td>
<td>37</td>
<td>80</td>
</tr>
<tr>
<td>PO₄-P (mg L⁻¹)</td>
<td>540</td>
<td>29</td>
<td>68</td>
</tr>
<tr>
<td>TKN (mg L⁻¹)</td>
<td>250</td>
<td>110</td>
<td>140</td>
</tr>
<tr>
<td>NH₄-N (mg L⁻¹)</td>
<td>89</td>
<td>23</td>
<td>41</td>
</tr>
<tr>
<td>Ca²⁺ (mg L⁻¹)</td>
<td>790</td>
<td>75</td>
<td>150</td>
</tr>
<tr>
<td>Mg²⁺ (mg L⁻¹)</td>
<td>110</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>K⁺ (mg L⁻¹)</td>
<td>1,300</td>
<td>79</td>
<td>165</td>
</tr>
<tr>
<td>Na⁺ (mg L⁻¹)</td>
<td>3,300</td>
<td>760</td>
<td>470</td>
</tr>
</tbody>
</table>

**ECSB reactor performance**

The ECSB reactor demonstrated stable performance with TCOD removal efficiency between 70% and 90% while operating at OLR from 4 up to 18 kg m⁻³ d⁻¹ (Figure 2(a)). The TCOD removal efficiency increased from 77 ± 5 to 82 ± 3% (p = 0.0004) with increasing the degree of wastewater pre-acidification. The effluent SCOD remained equal to 190 ± 50 and 150 ± 40 mg L⁻¹, (p = 0.0076) respectively, corresponding to an SCOD removal efficiency of 96 ± 1% (Figure 2(b)). Effluent VFA concentrations remained low (70 ± 20 mg L⁻¹) even during reactor operation at high OLR (~18 kg m⁻³ d⁻¹), indicating that the anaerobic digestion process was stable and robust. Similarly, a UASB reactor fed with pre-acidified CWW recorded COD removal efficiency around 83% while operating at OLR from 5 to 20 kg m⁻³ d⁻¹ (Diamantis et al. 2014). When the pre-acidification process was omitted, COD removal decreased to 64% and the reactor encountered process instabilities (with effluent COD > 2 kg m⁻³) and major alkali consumption (Diamantis et al. 2014). The beneficial effects of a two-stage anaerobic digestion process for cheese whey, considering stability and efficiency at high OLR, were also reported by Goblos et al. (2008).

The production of biogas was linearly related (R² > 0.90) to the applied organic loading rate (Figure 2(c)). Indeed, a maximum biogas production rate of 7.5 m³ m⁻³ d⁻¹ was achieved, with a methane content of 77 ± 4%, during reactor operation at OLR higher than 15 kg m⁻³ d⁻¹. The corresponding biogas yield was equal to 0.41 ± 0.04 and 0.48 ± 0.06 m³ kg⁻¹ COD for the low and high degree of wastewater pre-acidification, respectively (p < 0.0001). Considering the biogas methane content and the respective COD removal efficiency, methane yield was determined equal to 0.37 ± 0.05 Nm³ kg⁻¹ COD removed which is within the theoretical range. Similar methane yield was recorded for a laboratory-scale UASB reactor treating pre-acidified CWW (Diamantis et al. 2014).

Considering the data provided in Figure 2, the anaerobic effluent quality was adversely affected when the ECSB reactor was operated with a low degree of wastewater pre-acidification. Under these conditions, the effluent TCOD concentrations were on average 1,400 ± 450 mg L⁻¹ (compared to 870 ± 250 mg L⁻¹ for the high degree of wastewater pre-acidification). Therefore, it is evident that a significant fraction of particulate COD was removed from the ECSB reactor, attributed to the growth and washout of acidogenic biomass.

**Calcium precipitation efficiency**

The concentrations of calcium in CWW varied between 96 and 180 mg L⁻¹ (Figure 3a), depending on the influent cheese whey and wastewater flow. Indeed, the daily influent Ca²⁺ load to the anaerobic treatment facility under consideration, ranged from 100 up to 400 kg Ca²⁺ d⁻¹ (see Supplementary Material). During the first period (low degree of wastewater pre-acidification), the concentrations of Ca²⁺ in the ECSB reactor effluent showed a considerable decrease (from 136 ± 20 to 91 ± 14 mg L⁻¹) (p < 0.0001) corresponding to Ca²⁺ removal efficiency between 26% and 58% (Figure 3b). The Ca²⁺ removal efficiency decreased to 15–33% when the applied OLR was higher than 15 kg m⁻³ d⁻¹. Similarly, the first compartment of a staged anaerobic bioreactor encountered lower calcium precipitation (as demonstrated by the higher sludge VSS content), since this compartment received the higher OLR and VFA concentrations (El-Mamouni et al. 1995). Furthermore, with increasing the OLR of a UASB reactor, from 0.65 to 1.60 kg COD kg⁻¹ VSS d⁻¹, the granular sludge calcium content even decreased from 4.3 to 1.5 g Ca²⁺ kg⁻¹ TSS (Kosaric et al. 1990).

During the second study period (high degree of wastewater pre-acidification), the Ca²⁺ removal efficiency decreased from an average 35 ± 11% (during the first period) to 25 ± 14% (p = 0.018). Kosaric et al. (1990) reported calcium accumulation onto a UASB reactor granular sludge (from 1.1 to 4.3 g Ca²⁺ kg⁻¹ TSS) while treating a pre-acidified wastewater with an influent Ca²⁺ concentration 100 mg L⁻¹. Similarly, calcium removal efficiency
of 18% was recorded while treating fresh landfill leachate with an influent calcium content of 200 mg L\(^{-1}\) (Liu et al. 2014). Finally, the removal of Ca\(^{2+}\) in full-scale UASB reactors treating dairy wastewater was reported between 30% and 40% with influent Ca\(^{2+}\) concentrations between 130 and 180 mg L\(^{-1}\) (Manas et al. 2015).

**Granular sludge properties**

The anaerobic sludge was characterized by a TSS content between 65 and 75 kg m\(^{-3}\) and a VSS percentage of 82–87% of TSS. No decrease of VSS content was recorded during the study. In a previous work, during leachate
treatment, an expanded granular sludge bed (EGSB) reactor encountered sludge cementation and the density of anaerobic granules increased from 1.05 to 1.20 kg L\(^{-1}\) while the VSS content decreased from 70 to 60% (Liu et al. 2014). In this case, the influent Ca\(^{2+}\) concentration was 200 mg L\(^{-1}\).

In full-scale UASB reactors treating dairy wastewater having similar Ca\(^{2+}\) content (130–180 mg L\(^{-1}\)) the VSS percentage of the anaerobic granular sludge was as low as 20–30% of the TSS (Manas et al. 2017). Obviously this was not the case with the anaerobic granular sludge of our study.

The SMA of the anaerobic sludge samples increased during both experimental periods (Table 2). Indeed, a 50% (from 1.17 to 1.76 kg COD-CH\(_4\) kg\(^{-1}\) VSS d\(^{-1}\)) and an additional 45% (from 1.76 to 2.55 kg COD-CH\(_4\) kg\(^{-1}\) VSS d\(^{-1}\)) of sludge metabolic activity was recorded by the end of period I and II, respectively. Similarly, the SMA of anaerobic granular sludge from a UASB reactor increased from 0.26 to 1.10 kg COD-CH\(_4\) kg\(^{-1}\) VSS d\(^{-1}\) while treating a synthetic non-acidified wastewater with an influent calcium concentration of 150 mg L\(^{-1}\) (Yu et al. 2001). The SMA, however, decreased from 1.10 to 0.60 kg COD-CH\(_4\) kg\(^{-1}\) VSS d\(^{-1}\) with increasing the wastewater calcium concentration from 150 to 800 mg L\(^{-1}\) (Yu et al. 2001). In the latter case, the authors reported complete cementation of the anaerobic sludge at the bottom of the UASB reactor.

The anaerobic granular sludge from the ECSB reactor revealed high concentrations of iron and sulfur, followed by phosphorus, potassium and calcium (see Table 2). Surprisingly, the concentrations of Ca and P in the anaerobic granular sludge remained constant at 8 and 12 g kg\(^{-1}\) TSS, respectively, indicating that neither calcium carbonate nor calcium phosphate were precipitated onto the anaerobic granules (van Langerak et al. 1998; Liu et al. 2014). In studies with major sludge calcium precipitation, the granular sludge was characterized by Ca\(^{2+}\) content between 53 and 66 g kg\(^{-1}\) TSS (Liu et al. 2014) and phosphorus up to 70–190 g TP kg\(^{-1}\) TS (van Langerak et al. 1998).

### Table 2  Chemical and biological characteristics of anaerobic granular sludge samples obtained from the ECSB reactor by the end of period I and II and the seed sludge, respectively

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Seed sludge</th>
<th>Period I</th>
<th>Period II</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (kg m(^{-3}))</td>
<td>65.1</td>
<td>64.8</td>
<td>52.6</td>
</tr>
<tr>
<td>VSS (%)</td>
<td>81.9%</td>
<td>82.4%</td>
<td>87.2%</td>
</tr>
<tr>
<td>SMA (kg COD-CH(_4) kg(^{-1}) VSS d(^{-1}))</td>
<td>1.17 ± 0.1</td>
<td>1.76 ± 0.1</td>
<td>2.55 ± 0.0</td>
</tr>
<tr>
<td>Trace elements (g kg(^{-1}) TSS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>19</td>
<td>43</td>
<td>24</td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td>17</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>Phosphorus-total (P)</td>
<td>13</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>11</td>
<td>9.6</td>
<td>9</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>9.1</td>
<td>8.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.5</td>
<td>1.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>1.8</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Aluminum (Al)</td>
<td>2.0</td>
<td>0.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>0.054</td>
<td>0.170</td>
<td>0.071</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>0.010</td>
<td>0.045</td>
<td>0.012</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.052</td>
<td>0.011</td>
<td>0.024</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.027</td>
<td>0.010</td>
<td>0.029</td>
</tr>
<tr>
<td>Molybdenum (Mo)</td>
<td>0.018</td>
<td>0.019</td>
<td>0.013</td>
</tr>
</tbody>
</table>

SMA – Specific Methanogenic Activity.

### Implications for the anaerobic treatment of calcium-rich wastewaters

The calcium present in CWW precipitates with the carbon dioxide (carbonate ions) produced during the anaerobic digestion process, to form calcium carbonate.

![Figure 3](http://iwaponline.com/wst/article-pdf/78/9/1893/513717/wst078091893.pdf)
Calcium precipitation may occur either in the bulk liquid, on the surface of anaerobic granular sludge or within the core of the granules. The degree of wastewater pre-acidification affects the location of calcium precipitates. For completely acidified wastewaters, calcium precipitates on the outer part of anaerobic granular sludge (van Langerak et al. 1998). This results in mass transfer limitations and may decrease the sludge methanogenic activity (van Langerak et al. 1998). The SMA of a UASB reactor granular sludge, decreased from 1.96 to 0.61 g COD·CH₄ g⁻¹ VSS d⁻¹ within 180 d while treating acetate with an influent calcium concentration of 800 mg L⁻¹ (Yang et al. 2010). The anaerobic treatment of non-acidified wastewaters, by contrast, resulted in calcium precipitation inside the core of anaerobic granular sludge (Batstone et al. 2002). Internal calcium precipitation did not significantly affect sludge metabolic activity (van Langerak et al. 1998).

Based on the results of this study, the ECSB reactor displayed negligible Ca²⁺ accumulation onto the anaerobic granular sludge, despite that Ca²⁺ removal efficiency ranged from 10 to 50%. Therefore, it is speculated that calcium precipitation occurs in the bulk liquid and the formed crystals are subsequently washed-out of the bioreactor due to the high upflow velocity applied. Bulk liquid precipitation is generally favored inside the external circulation reactor due to anaerobic effluent alkalinity and biogas (CO₂) recirculation combined with NaOH supplementation. Similarly, van Langerak et al. (1997) reported high Ca²⁺ removal efficiency (up to 95%) using an external crystallization reactor with biogas and anaerobic effluent recirculation.

**CONCLUSIONS**

The ECSB reactor was efficiently operated long-term (2 years) for the anaerobic treatment of cheese industry wastewater. The degree of wastewater pre-acidification consisted an important parameter for process optimization. Over the OLR range examined, the ECSB reactor achieved a total COD removal efficiency equal to 75 ± 2 and 82 ± 3% for low (25%) and high degree (40%) of wastewater pre-acidification, respectively. Soluble COD removal remained >96% during the whole study period. Calcium precipitation varied between 10% and 50% and this pattern was affected by the degree of wastewater pre-acidification. The ECSB reactor is suitable for high-rate anaerobic treatment of calcium-rich wastewaters.

**ACKNOWLEDGEMENTS**

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