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 m^3) 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 $CaCO_3$ precipitates in the bulk liquid of the external circulation tank and not the biomass present in the main reactor, and that the $CaCO_3$ 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

Vasileios Diamantis (corresponding author)
Alexander Aivasidis
Laboratory of Wastewater Management and Treatment Technologies, Department of Environmental Engineering,
Democritus University of Thrace,
Vas. Sofias 12, Xanthi,
Greece
E-mail: *bdiamant@env.duth.gr*

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.* 2015). 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.* 2013). 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 Ca^{2+} 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,

doi: 10.2166/wst.2018.406

besides biomass cementation, severe flux decline due to membrane scaling (You *et al.* 2006) enabling the operation at extremely low permeate flux (~2 L m^{-2} h^{-1}) (Al-Malack & Aldana 2016). 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 3×100 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

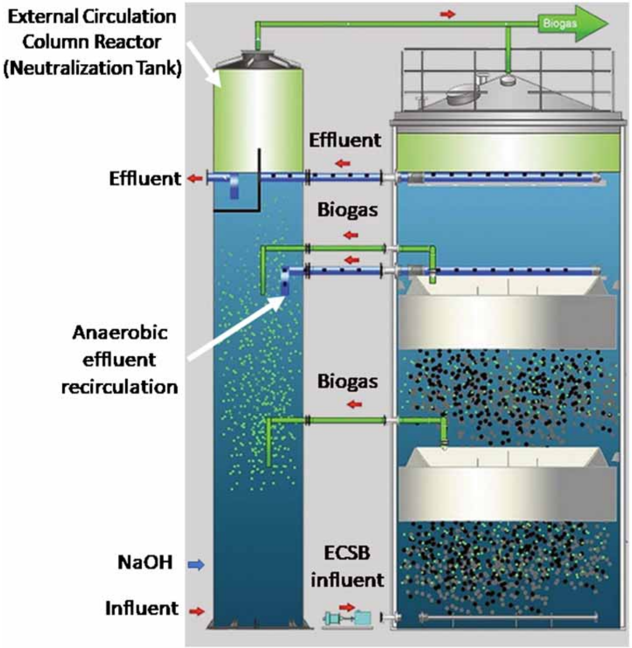

Figure 1 | Schematic representation of the external circulation sludge bed (ECSB) reactor used for this study.

$2{,}000 \pm 230\ m^3\ d^{-1}$ 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 Ca^{2+} 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 (Labinco, 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 CO_2. From the maximum methane production rate, the specific methanogenic activity (SMA) of the test biomass was calculated as g $COD\text{-}CH_4\ g^{-1}\ VSS\ d^{-1}$.

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 (PO_4^{3-}-P), total phosphorus, ammonia (NH_4^+-N), total Kjeldahl nitrogen (TKN), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+) and sodium (Na^+) according to *Standard Methods* (APHA 1998). The determination of soluble COD, PO_4^{3-}–P, NH_4^+–N and Ca^{2+} 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 HNO_3–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 $5{,}410 \pm 1{,}350\ mg\ L^{-1}$. The concentration of SCOD was $4{,}160 \pm 1{,}030\ mg\ L^{-1}$ 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 $136 \pm 20\ mg\ L^{-1}$, 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

Parameter	Cheese whey	Wastewater	Cheese industry wastewater
pH (–)	3.86	4.97	4.83
COD total (mg L^{-1})	53,850	2,500	5,410
COD soluble (mg L^{-1})	51,480	1,870	4,160
VFA (mg L^{-1} as acetic)	970	280	2,110
Total P (mg L^{-1})	530	37	80
PO_4-P (mg L^{-1})	540	29	68
TKN (mg L^{-1})	250	110	140
NH_4-N (mg L^{-1})	89	23	41
Ca^{2+} (mg L^{-1})	790	75	150
Mg^{2+} (mg L^{-1})	110	17	23
K^{+} (mg L^{-1})	1,300	79	165
Na^{+} (mg L^{-1})	3,300	760	470

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 ($R^2 > 0.90$) to the applied organic loading rate (Figure 2(c)). Indeed, a maximum biogas production rate of 7.5 m^3 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 m^3 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 Nm^3 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 ± 430 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 Ca^{2+} load to the anaerobic treatment facility under consideration, ranged from 100 up to 400 kg Ca^{2+} d^{-1} (see Supplementary Material). During the first period (low degree of wastewater pre-acidification), the concentrations of Ca^{2+} in the ECSB reactor effluent showed a considerable decrease (from 136 ± 20 to 91 ± 14 mg L^{-1}) ($p < 0.0001$) corresponding to Ca^{2+} removal efficiency between 26% and 58% (Figure 3b). The Ca^{2+} 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 Ca^{2+} kg^{-1} TSS (Kosaric *et al.* 1990).

During the second study period (high degree of wastewater pre-acidification), the Ca^{2+} removal efficiency decreased from an average 35 ± 11% (during the first period) to 23 ± 14% ($p = 0.018$). Kosaric *et al.* (1990) reported calcium accumulation onto a UASB reactor granular sludge (from 1.1 to 4.3 g Ca^{2+} kg^{-1} TSS) while treating a pre-acidified wastewater with an influent Ca^{2+} concentration 100 mg L^{-1}. Similarly, calcium removal efficiency

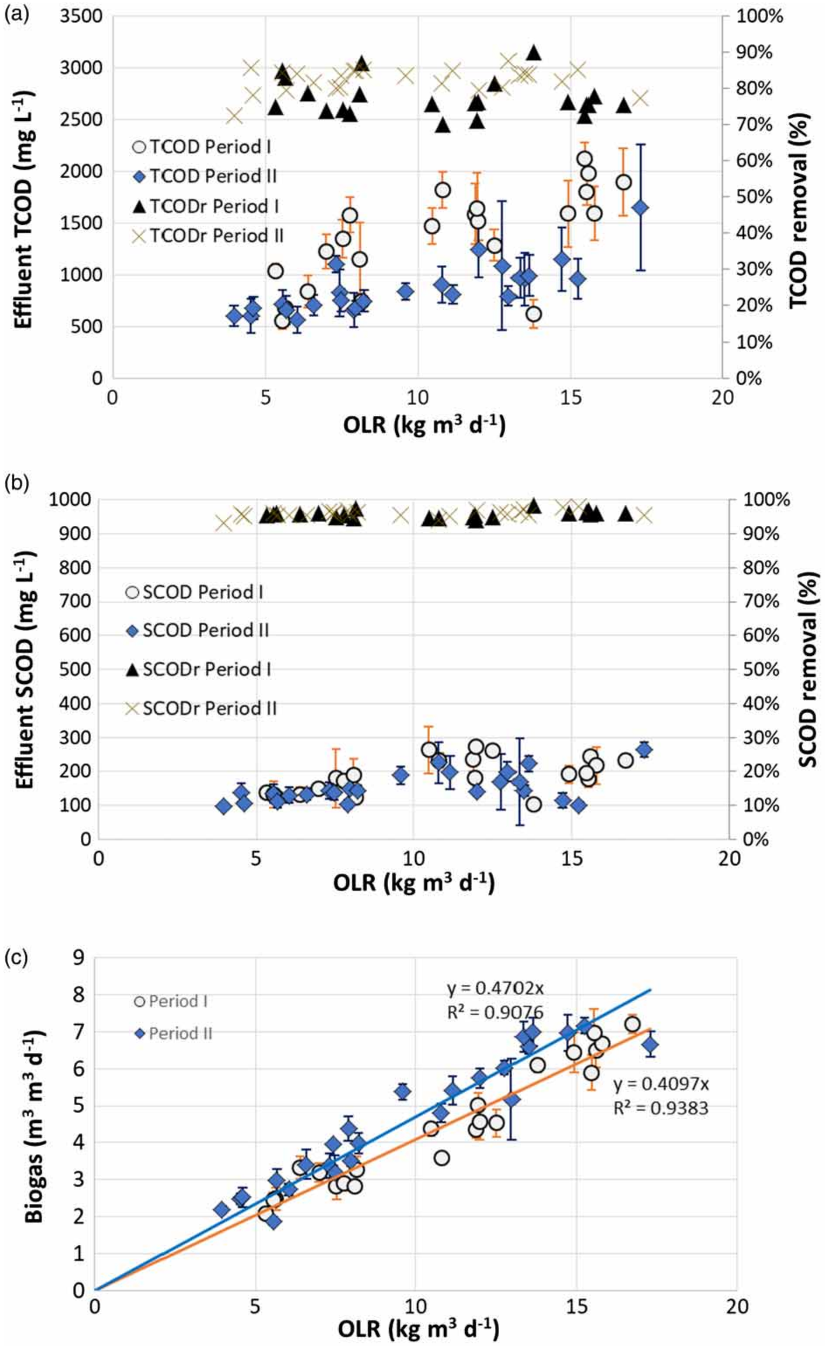

Figure 2 | Effect of organic loading rate on (a) effluent COD total (TCOD) concentration and removal efficiency (TCODr), (b) effluent COD soluble (SCOD) concentration and removal efficiency (SCODr) and (c) biogas production rate, during ECSB reactor operation under a low (Period I) and high (Period II) degree of wastewater pre-acidification.

of 18% was recorded while treating fresh landfill leachate with an influent calcium content of 200 mg L^{-1} (Liu *et al.* 2011). 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.* 2012).

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

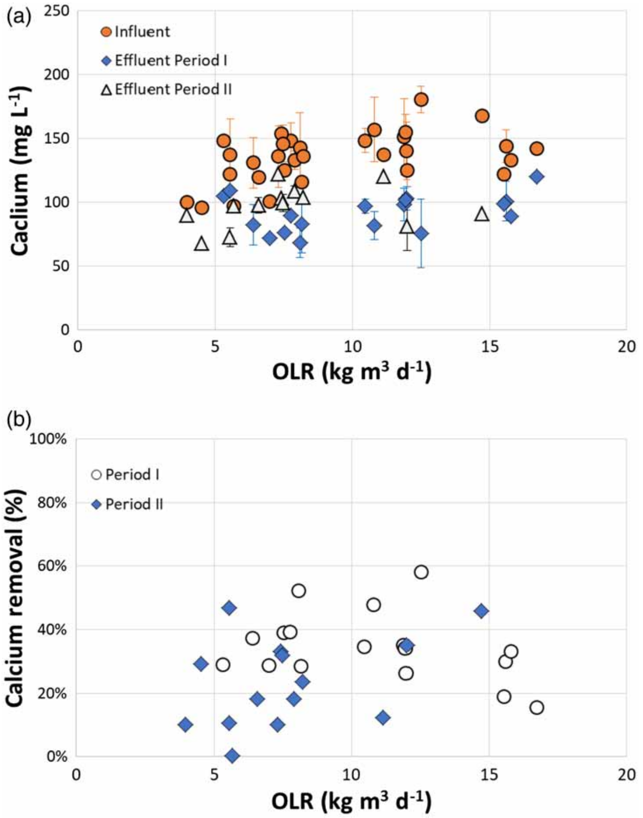

Figure 3 | Effect of organic loading rate on (a) Ca^{2+} concentrations and (b) Ca^{2+} removal efficiency, during ECSB reactor operation under a low (Period I) and high (Period II) degree of wastewater pre-acidification.

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

Parameter	Seed sludge	Period I	Period II
TSS (kg m^{-3})	65.1	64.8	52.6
VSS (%)	81.9%	82.4%	87.2%
SMA (kg COD-CH_4 kg^{-1} VSS d^{-1})	1.17 ± 0.1	1.76 ± 0.1	2.55 ± 0.0
Trace elements (g kg^{-1} TSS)			
Iron (Fe)	19	43	24
Sulfur (S)	17	35	22
Phosphorus-total (P)	13	12	12
Potassium (K)	11	9.6	9
Calcium (Ca)	9.1	8.1	8.3
Zinc (Zn)	0.5	1.2	2.5
Magnesium (Mg)	1.8	1.6	1.7
Aluminum (Al)	2.0	0.18	0.23
Nickel (Ni)	0.054	0.170	0.071
Cobalt (Co)	0.010	0.045	0.012
Cupper (Cu)	0.052	0.011	0.024
Manganese (Mn)	0.027	0.010	0.029
Molybdenum (Mo)	0.018	0.019	0.013

SMA – Specific Methanogenic Activity.

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.* 2011). 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.* 2012). 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% increase (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.* 2011). 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.* 2011) and phosphorus up to 70–190 g TP kg^{-1} TS (van Langerak *et al.* 1998).

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. Calcium carbonate

Table 3 | Previous studies reporting instabilities during anaerobic treatment of calcium-rich wastewaters (UASB – Upflow Anaerobic Sludge Blanket, MPAR – Multi Plate Anaerobic Reactor, AnMBR – Anaerobic Membrane Reactor)

Reactor	Remarks	Reference
UASB	Granular sludge samples from full-scale UASB reactors treating whey and dairy wastewater revealed high mineral content (70–80%) and low removal efficiency. Reactor re-seeding was also reported. The influent Ca^{2+} concentration ranged between 80 and 150 mg L^{-1} similar to our study. The respective effluent Ca^{2+} concentrations were between 75 and 90 mg L^{-1}.	Manas *et al.* (2012)
MPAR	With an influent calcium concentration of 1.6–2.4 g L^{-1} (due to lime addition), the anaerobic process was completely inhibited within 90 days. Calcium concentration in anaerobic sludge reached 40–50% TSS and VSS decreased to 20% TSS.	El-Mamouni *et al.* (1995)
UASB	Complete sludge bed cementation and clogging of feed manifolds was recorded in a 1,800 m^3 UASB reactor treating paper mill wastewater with a calcium concentration 400–700 mg L^{-1}. The concentration of calcium in anaerobic sludge reached 6% VS.	Batstone *et al.* (2002)
UASB	A synthetic wastewater supplemented with 800 mg L^{-1} Ca^{2+} showed major calcium precipitation (30–40% TS) on the surface and at the core of granular sludge when acidified and non-acidified wastewater was treated, respectively.	Yang *et al.* (2010)
AnMBR	Calcium carbonate precipitation resulted in scaling of anaerobic membrane bioreactors. Flux decline (77%) was far more severe compared to membrane fouling. Influent Ca^{2+} was maintained between 50 and 350 mg L^{-1}. The respective concentrations in the permeate were 20 and 80 mg L^{-1}. Calcium carbonate crystals were detected on the membrane surface.	You *et al.* (2006)

(and especially calcite) is the most common precipitate during anaerobic treatment of calcium-rich wastewaters (e.g. Yu *et al.* 2001; Batstone *et al.* 2002; Yang *et al.* 2010) and may cause major process instabilities (Table 3).

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-CH_4 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 Ca^{2+} accumulation onto the anaerobic granular sludge, despite that Ca^{2+} 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 (CO_2) recirculation combined with NaOH supplementation. Similarly, van Langerak *et al.* (1997) reported high Ca^{2+} 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

The financial support of TYRAS S.A. is highly acknowledged. Special thanks to HydroThane for the permission to publish data from the full-scale facility.

REFERENCES

Aivasidis, A. & Diamantis, V. 2005 Biochemical reaction engineering and process development in anaerobic wastewater treatment. *Advances in Biochemical Engineering and Biotechnology* **92**, 49–76.

Al-Malack, M. H. & Aldana, G. R. 2016 Performance of an anaerobic immersed membrane bioreactor (AnIMBR) treating synthetic dairy wastewater. *Desalination and Water Treatment* **57**, 22200–22211.

APHA/AWWA/WEF 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Batstone, D. J., Landelli, J., Saunders, A., Webb, R. I., Blackall, L. L. & Keller, J. 2002 The influence of calcium on granular sludge in a full-scale UASB treating paper mill wastewater. *Water Science and Technology* **45** (10), 187–193.

Carvalho, F., Prazeres, A. R. & Rivas, J. 2013 Cheese whey wastewater: characterization and treatment. *Science of the Total Environment* **445–446**, 385–396.

Chatzipaschali, A. A. & Stamatis, A. G. 2012 Biotechnological utilization with a focus on anaerobic treatment of cheese whey: current status and prospects. *Energies* **5**, 3492–3525.

Danalewich, J. R., Papagiannis, T. G., Belyea, R. L., Tumbleson, M. E. & Raskin, L. 1998 Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal. *Water Research* **32**, 3555–3568.

Demirel, B., Yenigun, O. & Onay, T. T. 2005 Anaerobic treatment of dairy wastewaters: a review. *Process Biochemistry* **40**, 2583–2595.

Diamantis, V., Melidis, P. & Aivasidis, A. 2006 Continuous determination of volatile products in anaerobic fermenters by on-line capillary gas chromatography. *Analytica Chimica Acta* **573–574**, 189–194.

Diamantis, V., Kapagiannidis, A. G., Ntougias, S., Tataki, V., Melidis, P. & Aivasidis, A. 2014 Two-stage CSTR-UASB digestion enables superior and alkali addition-free cheese whey treatment. *Biochemical Engineering Journal* **84**, 45–52.

EI-Mamouni, R., Guiot, S. R., Mercier, P., Sail, B. & Samson, R. 1995 Liming impact on granules activity of the multiplate anaerobic reactor (MPAR) treating whey permeate. *Bioprocess Engineering* **12**, 47–53.

Ghaly, A. E., Ramkumar, D. R., Sadaka, S. S. & Rochon, J. D. 2000 Effect of reseeding and pH control on the performance of a two-stage mesophilic anaerobic digester operating on acid cheese whey. *Canadian Agricultural Engineering* **42** (4), 173–183.

Goblos, S., Portoro, P., Bordas, D., Kalman, M. & Kiss, I. 2008 Comparison of the effectiveness of two-phase and single-phase anaerobic sequencing batch reactors during dairy wastewater treatment. *Renewable Energy* **33**, 960–965.

Kosaric, N., Blaszczic, R., Orphan, L. & Valladares, J. 1990 The characteristics of granules from upflow anaerobic sludge blanket reactors. *Water Research* **24**, 1473–1477.

Liu, J., Hu, J., Zhong, J., Luo, J., Zhao, A., Liu, F., Hong, R., Qian, G. & Ping Xu, Z. 2011 The effect of calcium on the treatment of fresh leachate in an expanded granular sludge bed bioreactor. *Bioresource Technology* **102**, 5466–5472.

Malaspina, F., Cellamare, C. M., Stante, L. & Tilche, A. 1996 Anaerobic treatment of cheese whey with a downflow-upflow hybrid reactor. *Bioresource Technology* **55**, 131–139.

Manas, A., Sperandio, M., Decker, F. & Biscans, B. 2012 Location and chemical composition of microbially induced phosphorus precipitates in anaerobic and aerobic granular sludge. *Environmental Technology* **33**, 2195–2209.

Marti, N., Bouzas, A., Seco, A. & Ferrer, J. 2008 Struvite precipitation assessment in anaerobic digestion processes. *Chemical Engineering Journal* **141**, 67–74.

Montastruc, L., Azzaro-Pantel, C., Biscans, B., Cabassud, M. & Domenech, S. 2003 A thermochemical approach for calcium phosphate precipitation modelling in a pellet reactor. *Chemical Engineering Journal* **94**, 41–50.

Van Langerak, E. P. A., Hamelers, H. V. M. & Lettinga, G. 1997 Influent calcium removal by crystallization reusing anaerobic effluent alkalinity. *Water Science and Technology* **36** (6–7), 341–348.

Van Langerak, E. P. A., Gonzalez-Gil, G., van Aelst, A., van Lier, J. B., Hamelers, H. V. M. & Lettinga, G. 1998 Effects of high calcium concentration on the development of methanogenic sludge in upflow anaerobic sludge bed (UASB) reactors. *Water Research* **32**, 1255–1263.

Van Langerak, E. P. A., Ramaekers, H., Wiechers, J., Veeken, A. H. M., Hamelers, H. V. M. & Lettinga, G. 2000 Impact of location of $CaCO_3$ precipitation on the development of intact anaerobic sludge. *Water Research* **34**, 437–446.

Van Lier, J. B., van der Zee, F. P., Frijters, C. T. M. J. & Ersahin, M. E. 2015 Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. *Reviews in Environmental Science and Biotechnology* **14**, 681–702.

Vidal, G., Carvalho, A., Mendez, R. & Lema, J. M. 2000 Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. *Bioresource Technology* **74**, 231–239.

Yang, S., He, Y., Liu, Y., Chou, C., Zhang, P. & Wang, D. 2010 Effect of wastewater composition on the calcium carbonate precipitation in up*fl*ow anaerobic sludge blanket reactors. *Frontiers in Environmental Science Engineering China* **4** (2), 142–149.

You, H. S., Huang, C. P., Pan, J. R. & Chang, S. C. 2006 Behavior of membrane scaling during crossflow filtration in the anaerobic MBR system. *Separation Science Technology* **41**, 1265–1278.

Yu, H. Q., Tay, J. H. & Fang, H. H. P. 2001 The roles of calcium in sludge granulation during UASB reactor start-up. *Water Research* **35**, 1052–1060.

First received 29 March 2018; accepted in revised form 7 September 2018. Available online 18 September 2018