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The treatment of cheese whey wastewater by sequential anaerobic and aerobic steps in a single digester at pilot scale

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ABSTRACT

The treatment of reconstituted whey wastewater was performed in a 400 L digester at 20 °C, with an anaerobic digestion step, followed by a step of aerobic treatment at low oxygen concentration in the same digester. In a first set of 48 cycles, total cycle time (T_C) of 2, 3 and 4 days were tested at varying organic loading rates (OLR). The COD removal reached 89 ± 4, 97 ± 3 and 98 ± 2% at T_C of 2, 3 and 4 days and OLR of 0.56, 1.04 and 0.78 gCOD L^{-1} d^{-1}, respectively. The activity of the biomass decreased for the methanogenic population, while increasing by 400% for the acidogens, demonstrating a displacement in the predominant trophic group in the biomass bed. A second set of 16 cycles was performed with higher soluble oxygen concentration in the bulk liquid (0.5 mg L^{-1}) during the aerobic treatment at a T_C of 2 days and an OLR of 1.55 gCOD L^{-1} d^{-1}, with a soluble COD removal of 88 ± 3%. The biomass specific activities showed a compartmentalization of the trophic group with methanogenic activity maintained in the biomass bed and a high acidogenic activity in the suspended flocs.

1. Introduction

Whey is the liquid residue obtained when casein and fat are separated by coagulation from the milk. Whey contains lactose (70–75%) and soluble proteins (10–15%) which results in a high chemical oxygen demand (COD) (50–70 g L^{-1}). At large milk processing plants, whey is usually dried and used as feedstock for animal feeding or more recently by the agrifood and pharmaceutical industries. However, at small-scale milk farm or cheese producers, which are common in isolated rural areas, whey is not recuperated and has to be treated along with the other generated wastewaters from the installation, since the small quantity produced does not justify the significant cost of the equipment needed for the preparation of whey powder. The final COD after mixing of the whey wastewater with the washing waters results in a diluted effluent (2–4 gCOD L^{-1}) requiring treatment before discharge. Since suggested treatments are also expensive, there is a need for a cost-effective option for the treatment of whey wastewater at small-scale facilities.

Many different biological processes have been tested for the treatment of cheese whey wastewaters, as extensively reviewed by Demirel et al. (2005). Studies on the treatment of cheese whey wastewaters have been initially performed with single phase anaerobic digestion systems (Barford et al., 1986; Yan et al., 1989). The anaerobic digestion of whey wastewaters can be problematic for the state-of-health of the biomass in the digester. In effect, since whey is rich in lactose, it tends to acidify rapidly resulting in a pH drop (pH 4). At that pH the concentration of the non-dissociated VFAs in the bulk liquid is too high inhibiting the methanogens and destabilizing the digester (Yan et al., 1993). Acidification of the whey wastewater also leads to the use of buffering in the digester. The use of lime, $Ca(OH)_2$, is not suggested since it forms calcium precipitate in the biomass (El-Mamouni et al., 1995). The pH can be adjusted with bicarbonate (Cocci et al., 1991; Ratusznei et al., 2003) or NaOH (Yang et al., 2003). Long retention times (over 5 days) have also been used to minimize the acidification effect (Lo and Liao, 1986). Nevertheless, some studies have reported good operation of the digesters without any pH adjustment (Kalyuzhnyi et al., 1997; Malaspina et al., 1995).

Another negative impact on the biomass is the destruction of granules that can be observed along with a strong build up of visquous material resulting in a bad settling of the solids and subsequent washout of the biomass from the digester. This has led to different strategies to minimize the acidification of the wastewater under treatment and the biomass washout, such as the use of a single digester with suspended or fixed biomass (Kalyuzhnyi et al., 1997; Ratusznei et al., 2003; Rodgers et al., 2004). Two-phase anaerobic processes, where the acidogenic and methanogenic

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digestion are conducted in two separate digesters were also investigated (Yilmazer and Yenigün, 1999; Yang et al., 2003; Saddoud et al., 2007).

The complete treatment of whey wastewaters commonly requires two steps, the anaerobic degradation of the main fraction of organic matter, then a polishing step of the partially treated wastewaters by aerobic treatment to lower the final organic load of the effluent so it can meet the discharge requirements. The aerobic polishing step can be provided by aerated ponds (Cocci et al.,1991; Monroy et al., 1995). In these studies, the anaerobic digestion and aerobic polishing were performed in two separate units. Small-scale industries can not afford these systems and are thus looking at alternatives involving anaerobic digesters. The use of a single digester for the main anaerobic treatment and the subsequent aerobic polishing could match their financial capacity. The sequential coupling of anaerobic and aerobic degradation in a single digester was proposed as a mean for the small-scale cheese producers to treat their wastewaters in an economical way.

The sequential anaerobic and aerobic treatment of dairy wastewaters have been reported previously by Malaspina et al. (1995) with a SBR reactor treating cheese whey wastewater after prior anaerobic digestion in a downflow–upflow hybrid reactor. The SBR was used in anoxic and aerobic cycles within batches of 24 h, and achieved COD removal of 88–94% with residual sCOD concentration between 137 and 375 mg L^{-1}. Their main objective was to reduce the high nitrogen and phosphorous concentration still present in the anaerobically treated whey wastewater, and different food to biomass ratio resulted in 66–93% nitrogen and 35–93% phosphorous removal.

Most of the reported studies in whey wastewater treatment were performed at mesophilic (35 °C) or thermophilic (55 °C) conditions, although psychrophilic digestion can result in lower treatment costs and become more appropriate for small-scale cheese producers. There is a need for further optimization and advancement of psychrophilic digestion, in order to increase the applicability of the process and to maximize the understanding of the microbiology of the process (McHugh et al., 2006). Preliminary studies of the anaerobic and aerobic sequential treatment of whey wastewater at psychrophilic temperature in a single digester was demonstrated in 0.5 L SBR (Frigon et al., 2007). The SBR was operated at cycles of 48 h, with different levels of aeration after an initial anaerobic incubation of 30 h. An addition of 54 mg O_2/$gCOD_{in}$ over 16 h showed the best performance with a sCOD removal reaching 99% and a residual sCOD of 104 ± 22 mg L^{-1}. This study aims at evaluating the potential of performing psychrophilic anaerobic digestion of most of the biodegradable material contained in the whey wastewaters, then completing the treatment with an aerobic polishing sequence, in a single digester, in order to limit investment costs. The final goal was to provide small-scale cheese producers with an efficient and economical process for treating their wastewaters.

2. Methods

2.1. Description of the SBR

The pilot-scale digester had a diameter of 0.57 m, and a height of 1.57 m for a total volume of 445 L and a working volume of 400 L (Fig. 1). The headspace was kept minimal using a floating cover. Several sampling ports were installed along the height of the reactor, at 0.04, 0.25, 0.36 m from the bottom. The oxygen probe and pH probe were connected to an Accumet Reader (model 825MP, Fisher, Pittsburgh, USA) and installed when required in the 0.24 and 0.36 m port. Biogas production was measured with a Wet Test gas meter (model L-1, Wohlgroth, Schwerzenbach, Switzerland). The mixing was performed by biogas recirculation through four pressure released valves (1/8 psi) (model 8C4, serie C, Swagelock, USA) inserted at the bottom of the SBR, and a peristaltic pump delivering a flowrate of 1.5 L min^{-1} (Fig. 1).

The inlet and outlet tubing used for filling the digester with the reconstituted wastewater and drawing the treated effluent from the digester was maintained 5 cm under the water level inside the digester with a float. The tubings were connected to a centrifuge pump working at 11 L min^{-1}.

2.2. Operation of the SBR

The digester was operated in batch mode. Different total cycle times (T_C) were tested, from 48 h, as previously tested in small-scale SBR (Frigon et al., 2007), to 3 and 4 days. A batch, considered as a "cycle" in the text, consisted of the following steps: filling of the digester, anaerobic digestion step, aerobic polishing step, settling, withdrawing of the treated wastewater. During the 48, 72 or 96 h batch cycle, the time for each step was as follows: filling of the reconstituted wastewater was done in 1 h. The anaerobic step lasted for 26, 50 or 74 h, followed by 16 h for the aerobic step. The aeration and mixing were stopped at time 43, 67 or 91 h for 4 h of settling followed with 1 h for withdrawing of the treated effluent.

The digester was operated in a temperature controlled pilot plant and the temperature was maintained around 20 °C. The digester temperature was presumed to be the same as for the room. Also, the temperature was measured occasionally on effluent samples (data not shown) and confirmed the results (variation between 18 and 22 °C).

The operation of the digester was divided into two phases, depending on the intensity of aeration during the aerobic step, as described below. Phase 1 consisted in 48 cycles of operation at a T_C of mainly 2, 3 or 4 days (Fig. 2). Phase 2 consisted of 16 cycles with a fixed T_C of 48 h and a fixed OLR of 1.5 gCOD L^{-1} d^{-1}.

The volumetric exchange ratio (VER), e.g. the amount of fresh wastewater added divided by the total working volume of the digester, also varied during Phase 1 (Fig. 2). In effect, the ratio was kept at 0.25 (100 L of fresh wastewater) during the first 20 cycles, but the final objective was to obtain a VER of 0.75, e.g. 300 L of fresh wastewater added in the digester for each cycle, in order to represent the operating conditions of the unit at the cheese producer. The VER was thus increased at 0.50 between cycles #21 and #27, and maintained at 0.75 from cycles #28 to #48. For a VER of 0.25, 0.50 and 0.75, an additional 60, 160 and 260 L of tap water was thus added to the 40 L of reconstituted wastewater for the feeding of the digester, respectively. An equivalent amount of treated wastewater was removed from the digester prior to the addition of the fresh wastewater.

In addition to the variation of the T_C and the VER, the OLR was also varied during Phase 1 (Fig. 2). The OLR was adjusted by adding different amounts of whey during the preparation of the reconstituted wastewater. The OLR was adjusted at around 0.5, 0.75, 1.0 and 1.5 gCOD L^{-1} d^{-1} by adding 0.3, 0.6, 0.9 and 1.2 kg of whey powder in the reconstituted wastewater, respectively. The OLR was calculated using the total COD of the added reconstituted whey wastewater since part of the whey powder was not completely solubilized prior to addition, even after 16 h of mixing.

During Phase 1, the aerobic step was performed by injecting ambiant air through a membrane diffusor (model "Simple disc", Produits Étang, Knowlton, Canada) located at the bottom center of the digester, using a peristaltic pump working at 1.35 L min^{-1}. During Phase 2, the aerobic step was performed using a target dissolved oxygen concentration in the bulk liquid of the digester. Hence, the peristaltic pump was coupled to a controller, and con-

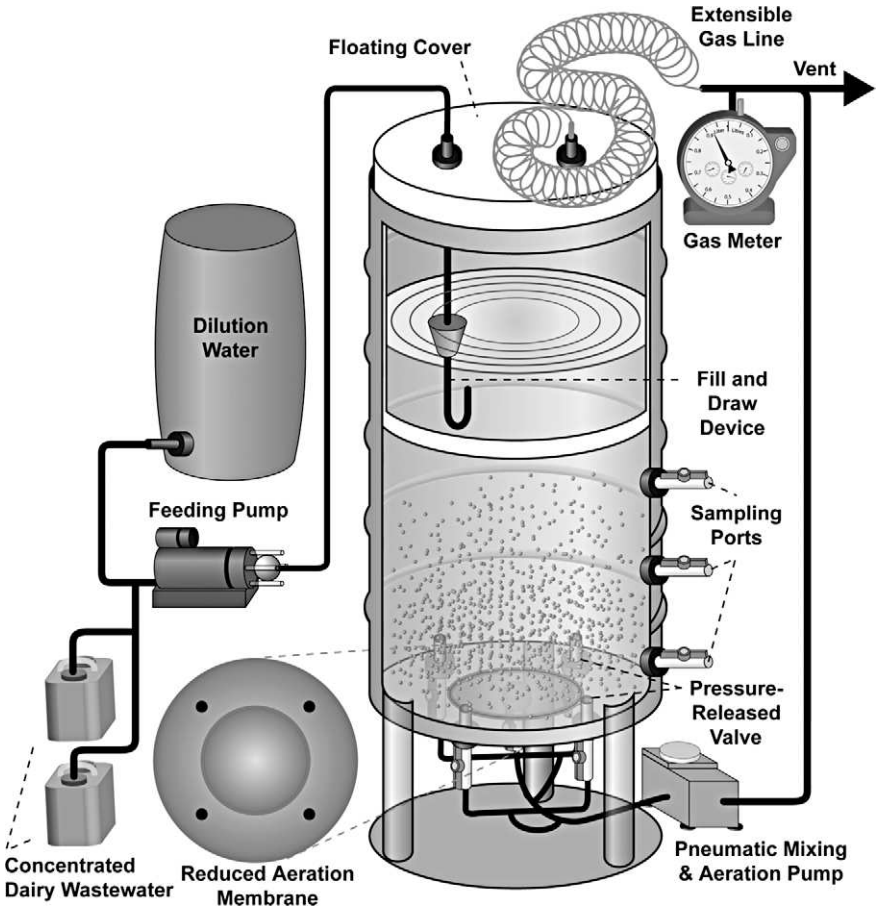

Fig. 1. Schematic of the pilot-scale digester.

nected to a dissolved oxygen probe. The pump was activated, and ambiant air was delivered through the membrane diffusor, when the dissolved oxygen concentration dropped below 0.5 mg L^{-1} in the bulk liquid of the digester.

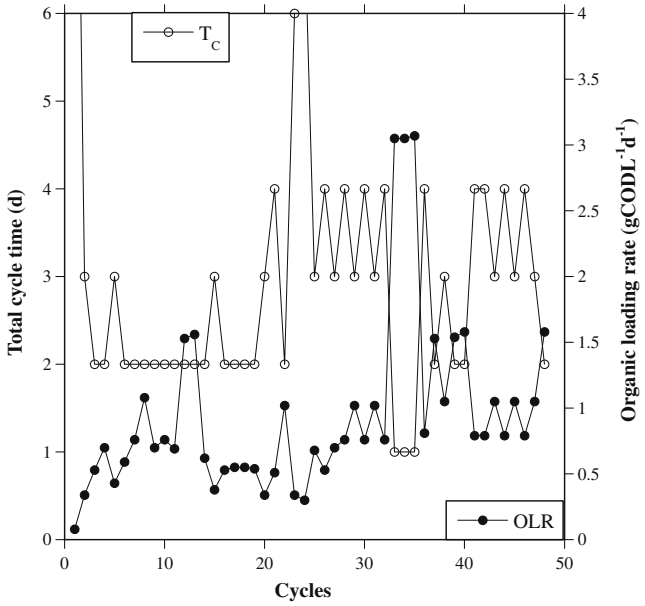

Fig. 2. Variation of the T_C and OLR during Phase 1 of operation.

The digester was initially inoculated with 6.7 L of an active anaerobic biomass (110 g VSS L^{-1}) and 4 L of an aerobic biomass (3.2 g VSS L^{-1}). At day 31 of operation, 2.3 L of the same anaerobic biomass was added to compensate for the biomass loss at the beginning of the operation of the SBR.

2.3. Preparation of the reconstituted whey wastewater

The cheese-whey used for this study was reconstituted from whey powder (Agropur, Notre-Dame-du-Bon-Conseil, Qc, Canada). The reconstituted whey wastewater was prepared 16 h in advance for better solubilization of the whey powder and kept at 4 °C, using the same basic recipe as for the previous study (Frigon et al., 2007). Bicarbonate was supplemented to the reconstituted wastewater in order to maintain proper pH during the reaction. The addition was performed initially, although adjusted alkalinity supplementation can also be performed (Mockaitis et al., 2006). The recipe for the reconstituted wastewater prepared for an OLR of 1.5 gCOD L^{-1} d^{-1} consisted in 1.2 kg of whey powder; 1.4 kg of $NaHCO_3$ and 1.4 kg of $KHCO_3$ dissolved in 40 L of tap water.

The reconstituted wastewater was analyzed for more parameters during the kinetics assays at the end of Phase 2, and contained (mg L^{-1}): COD, 3900 ± 10; lactose, 2807 ± 10; BOD, 2700; total Kjeldahl nitrogen, 95 ± 20; total phosphorous, 20 ± 5; sodium, 34 ± 2; potassium, 80 ± 2; sulfate, 8 ± 2. The COD:N and COD:P ratio were 100:2.4 and 100:0.5, respectively, which was insufficient for complete aerobic treatment (preferred ratio of 100:5 and 100:2) but satisfactory for anaerobic degradation. The experiments were conducted without nutrients addition, contributing to cost reduction.

2.4. Analytical methods

Liquid samples (50 mL) were taken from the sampling ports with 60 mL syringes, or directly from the effluent at the end of the degradation step, and analyzed for pH, sCOD and VFA. Volatile suspended solids (VSS) were also determined periodically from 10 mL samples from each sampling port to determine the amount of biomass in the digester. The pH was measured on an Accumet AP61 portable pH meter equipped with a micro probe (Fisher, Fairlawn, USA) directly on the sample, within 1 min of sampling. The sCOD and the VSS were determined according to Standard Methods (APHA et al., 1995). The VFA were determined from a 350 μL supernatant sample mixed with an internal standard (iso-butyric acid 300 mg L^{-1}). One volume of this sample was mixed with one volume of formic acid 6% (w/v) and 1 μL of this mixture was injected into a Perkin–Elmer Sigma 2000 (Norwalk, USA) gas chromatograph equipped with a flame-ionization detector (FID) and a 76 cm glass column (4 mm ID) filled with Carbowax 20 M (0.3%) and phosphoric acid (0.1%) on 60/80 Carbopack C (Arcand et al., 1994). The samples were collected from the digester and filtered within 15 min of sampling. A standard solution containing 1000 mg L^{-1} of acetate, propionate and butyrate was similarly injected and used for VFA calculation.

Off-gas was analyzed for H_2, N_2, O_2, CH_4 and CO_2 content on a Agilent 6890 (Agilent Technologies, Wilmington, DE) gas chromatograph (GC) coupled to a thermal conductivity detector (TCD). A sample of 300 μL of the digester headspace was taken with a model 1750 gas-tight syringe (Hamilton, Reno, USA) and injected on a 11 m × 2 mm I.D. Chromosorb 102 packed column (Supelco, Bellafonte, PA). The column was heated at 35 °C for 7.5 min then raised to 100 °C at a rate of 75 °C/min, maintained for 6 min. Argon was used as the carrier gas. The injector and detector were maintained at 125 and 150 °C, respectively. Total Kjhedhal Nitrogen (TKN) (SM4500-NH3 B, D, H), phosphorous (SM4500-P, E) and biochemical oxygen demand (BOD) (SM5210B) were performed according to Standard Methods (1995).

2.5. Specific activity tests

Biomass specific activities for glucose, acetate, oxygen and hydrogen were determined in serum bottles by measuring the depletion rate of the given substrate, individually and under non-limiting conditions, as described in Arcand et al. (1994). The biomass is diluted into phosphate buffer (0.05 M) to a final concentration of around 5 g VSS L^{-1} for the liquid substrate assay and 1–2 g VSS L^{-1} for the gaseous substrate assay, then transferred into the serum bottles (triplicate). The substrate was injected at a concentration of 11 and 50 mmol L^{-1} for the glucose and acetate test, respectively. For the hydrogen test, the substrate is provided by pressurizing the headspace of the bottle at 20 psi with a gas mixture (80% H_2:20% CO_2) for a final H_2 concentration of 90 mmol L^{-1} in the headspace. The oxygen test was prepared similarly excepted that 15 mL of pure oxygen was added through the septum of the bottle, for a final O_2 concentration of 60 mmol L^{-1}. The residual concentration of the substrate is measured over time. At the end of the assay, the amount of substrate degraded is divided by the VSS concentration determined in each bottle, in order to obtain the activity, expressed in milligram of substrate degraded per gram of VSS (biomass) and per day.

3. Results and discussion

3.1. Digester performance during Phase 1

The operating conditions of the digester were varied during the 48 cycles of Phase 1. The T_C was set mainly at 2, 3 or 4 days, except during cycles #1 (11 days), #22 to #24 (7 days) and #33 to #35 (1 day). Different OLR were tested for each T_C. The results from Table 1 for a T_C of 2 days were averaged from cycles #7 to #11, #14 to #19, and #37 to #40 for the OLR of 0.72 ± 0.03, 0.56 ± 0.03 and 1.55 ± 0.02 gCOD L^{-1} d^{-1}, respectively. The initial pH was maintained around 7.2–7.4 for most of the cycles. The pH stayed around 7.0 after anaerobic and aerobic step for all cycles (Table 1). The residual sCOD after the anaerobic step increased in parallel with each increase in OLR, up to 736 ± 390 mg L^{-1} for an OLR of 1.55 gCOD L^{-1} d^{-1}. The corresponding sCOD removal were 79 ± 14, 62 ± 11 and 69 ± 18% at OLR of 0.56, 0.72 and 1.55 gCOD L^{-1} d^{-1}, respectively. The lower performance at the 0.72 gCOD L^{-1} d^{-1} OLR can be attributed to the fact that these cycles were performed at the beginning of the operation of the digester where important biomass washout occurred. The degradation of the reconstituted whey wastewater was pursued during the aerobic step, with a residual sCOD of 113 ± 37 mg L^{-1} at the lowest OLR (Table 1), for total COD removal of 89 ± 4, 70 ± 18 and 84 ± 7% at OLR of 0.56, 0.72 and 1.55 gCOD L^{-1} d^{-1}, respectively.

The results from Table 1 for a T_C of 3 days were averaged from cycles #25 to #27, and #29 to #38 for the OLR of 0.69 ± 0.01 and 1.04 ± 0.02 gCOD L^{-1} d^{-1}, respectively. The initial pH stayed around 7.2–7.4 for most of the cycles. The pH was maintained around 7.0 after anaerobic and aerobic step for all cycles (Table 1). The residual sCOD after the anaerobic step was not measured during those cycles. However, the measured concentration were at 112 ± 101 and 51 ± 56 mg L^{-1} after the aerobic step, for sCOD removal of 92 ± 7 and 97 ± 3%, at the 0.69 ± 0.01 and 1.04 ± 0.02 gCOD L^{-1} d^{-1} OLR, respectively.

The results from Table 1 for a T_C of 4 days were averaged from cycles #21 to #26, and #28 to #41 for the OLR of 0.52 ± 0.01 and 0.78 ± 0.02 gCOD L^{-1} d^{-1}, respectively. The initial pH stayed

Table 1
Operational parameters during Phase 1 for the different tested cycle times.

OLR (gCOD L^{-1} d^{-1})	pH			Residual sCOD (mgL^{-1})		
	In	An	Ae	In	An	Ae
T_C 2 days						
0.56 ± 0.03	7.7 ± 0.4	7.0 ± 0.1	7.0 ± 0.1	1051 ± 56	217 ± 137	113 ± 37
0.72 ± 0.03	7.3 ± 0.7	7.0 ± 0.1	7.0 ± 0.1	1200 ± 132	470 ± 186	373 ± 252
1.55 ± 0.02	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	2589 ± 493	736 ± 390	392 ± 94
T_C 3 days						
0.69 ± 0.01	7.2 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	1360 ± 185	ND	112 ± 101
1.04 ± 0.02	7.4 ± 0.4	7.1 ± 0.4	7.2 ± 0.3	1775 ± 606	ND	51 ± 56
T_C 4 days						
0.52 ± 0.01	7.3 ± 0.1	7.1 ± 0.1	7.1 ± 0.2	1544	ND	125
0.78 ± 0.02	7.3 ± 0.4	7.3 ± 0.1	7.4 ± 0.2	1984 ± 558	312 ± 200	33 ± 29

In: initial; An: after anaerobic step; Ae: after aerobic step.

around 7.2–7.4 for most of the cycles. The pH was maintained at the same level after anaerobic and aerobic step for all cycles (Table 1). The residual sCOD of 312 ± 200 mg L^{-1} after the anaerobic step during the higher applied OLR corresponded to a 84% COD removal. After the aerobic step, the combined COD removal reached 92 and 98 ± 2% for the OLR of 0.52 ± 0.01 and 0.78 ± 0.02 gCOD L^{-1} d^{-1}, respectively. The lowest residual sCOD concentration for the Phase 1 experiment was obtained at a T_C of 4 days at 33 ± 29 mg L^{-1}.

The different T_C and OLR tested during Phase 1 were compared as pairs of data with a *F* test to determine if their variances were equal or not. Then, the proper *t*-test (equal or unequal variances) was applied to each pair of data, in order to statistically assess their difference. The alpha was set at 0.1, thus any *P* value lower than 0.100 would qualify the tested pair of data (means of residual sCOD at respective T_C and OLR) as significantly different. For the T_C of 2 days, the residual sCOD were found not statistically different between the OLR, with *P* values from the *t*-tests of 0.257, 0.168 and 0.438 for the OLR of 0.56, 0.72 and 1.55 gCOD L^{-1} d^{-1}, respectively (Table 2). The addition of 1 day of T_C greatly improved the COD removal in the digester, with significantly different residual sCOD between the T_C of 2 and 3 days, at *P* values ranging from 0.001 to 0.089 when comparing the different OLR tested. The residual sCOD was however not statistically different between the OLR of 0.69 and 1.04 gCOD L^{-1} d^{-1} for a T_C of 3 days (*P* value = 0.282). A T_C of 4 days did not significantly improved the performance of the digester, compared to a T_C of 3 days, with *P* values ranging from 0.185 to 0.465 for the different OLR (Table 2). The residual sCOD was statistically different between the OLR of 0.52 and 0.78 gCOD L^{-1} d^{-1} for a T_C of 4 days (*P* value = 0.021). Thus, the biggest impact on the performance of the digester was the shift from a T_C of 2 to 3 days, and not from the applied OLR inside a T_C fixed at 2 or 3 days.

To our knowledge, only the work of McHugh et al. (2006) reported psychrophilic anaerobic digestion of whey wastewaters. In their study, a 4 L anaerobic digester was first operated at 20 °C and removed 77% of the COD at an OLR of 0.5 gCOD L^{-1} d^{-1} and an HRT of 48 h, somewhat lower than our 89% COD removal achieved for the same OLR. Then the OLR was increased by decreasing the HRT to 24 and 18 h, with COD removal ranging between 78 and 86%, which is higher than what was observed during our experiment at an T_C of 2 days. The increase in performance with shorter HRT in their anaerobic digester could be attributed to the type of digester used in their work (UASB in three section with sludge retention device) compared to our more rustic anaerobic SBR. The use of a sludge retention device (gas solid separator) in a UASB was also beneficial for Strydom et al. (1995) who reported COD removal of 90–97% at OLR of 0.82–6.11 gCOD L^{-1} d^{-1} although the residual COD was still considered too high for direct discharge (300–800 mg L^{-1}). The residual COD remained higher than the concentrations displayed by our SBR at similar OLR.

The residual sCOD obtained at T_C of 3 and 4 days from our SBR (33–51 mg L^{-1}) can be compared favorably with results from Mockaitis et al. (2006) who obtained 51 ± 11 and 33 ± 8 mg L^{-1} for OLR of 0.6 and 1.15 gCOD L^{-1} d^{-1} using a 5 L stirred anaerobic SBR operated at 8 h cycles with a VER of 0.4 and at 30 °C. Similarly, a residual COD of 137 mg L^{-1} was achieved in a downflow–upflow hybrid reactor where sequential anoxic and aerobic cycles (HRT 24 h) were applied as a post treatment after anaerobic digestion of high strength whey wastewater (68 gCOD L^{-1}) by Malaspina et al. (1995). Also, a large-scale SBR (22 m^3) was used to treat the treated dairy wastewater from an anaerobic filter, at OLR of 0.5–1.5 gCOD L^{-1} d^{-1}, achieving sCOD concentration of 20–200 mg L^{-1} in the effluent (Garrido et al., 2001). High COD removal is also possible at high OLR, as reported by Ramasamy et al. (2004) with a UASB operated at an OLR of 2.4–13.5 gCOD L^{-1} d^{-1} and a HRT varying between 3 and 12 h for a 96% COD removal. Hence, high COD removal and low residual COD concentration is attainable at psychrophilic conditions, although the HRT has to be increased compared to mesophilic conditions and/or high rate systems in order to obtain similar performance.

Table 2
P values from *t*-test performed between the different loading applied to the SBR during Phase 1.

	A	B	C	D	E	F	G
A		0.257	0.168	0.213	0.044	0.307	0.037
B			0.438	0.089	0.025	0.178	0.013
C				0.011	0.001	0.030	0.001
D					0.282	0.465	0.305
E						0.185	0.315
F							0.021
G							

The letters represent the different combination of T_C and OLR tested on the SBR: A (T_C 2; OLR 0.56), B (T_C 2; OLR 0.72), C (T_C 2; OLR 1.55), D (T_C 3; OLR 0.69), E (T_C 3; OLR 1.04), F (T_C 4; OLR 0.52), and G (T_C 4; OLR 0.78). OLR (gCOD L^{-1} d^{-1}).

3.2. Biomass volume

The instability of the digester performance for the first 10 cycles was related to the significant biomass washout from the digester, with an estimated 2.4 kg VSS of biomass remaining at cycle #11 from the 6.7 kg initially inoculated. This could be partly due to the normal washout of the acidogenic floc, which do not settle well, since around 80 g VSS of floating biomass were removed at the end of each cycle. Some of the more viscous material could also be related to exopolysaccharides, as reported by Malaspina et al. (1995) in their downflow–upflow hybrid reactor treating high strength cheese whey wastewater. Also, the aeration could have resulted in the degranulation of the anaerobic biomass. The resulting washout was thus greater than the biomass growth. An additional seeding of 2.2 kg of anaerobic biomass was performed after cycle #11. Common strategies to limit biomass washout, besides adding sludge retention device mentioned above, include two stage treatment (Yang et al., 2003); or a fixed film to maintain the biomass inside the digester (Ratusznei et al., 2003). A different strategy was used in our study, with an extended settling of the effluent at the end of the cycle, with recuperated sludge return, allowing for a more stable quantity of reactive biomass maintained in the digester, as shown by the similar amount of sludge bed found at cycle #41 (4.4 kg VSS). The biomass concentration was thus maintained over time, but not increased, in the digester.

3.3. Biomass specific activities

The activity of the biomass for specific substrates fluctuated greatly during the Phase 1 experiment. The activity on acetate, used as an estimation of the activity of acetoclastic methanogens, increased from the start-up to cycle #14, but remained at 114 ± 8 mgAc g VSS^{-1} d^{-1} at the end of Phase 1 (cycle #44), as shown in Table 3. The activity on hydrogen, used as an estimation for the activity of hydrogenotrophic methanogens, was high during the first cycles, then decreased sharply and remained very low from cycle #29 to #44 (65 ± 3 mgH_2 g VSS^{-1} d^{-1}). The activity on oxygen, used as an estimation for the general activity of aerobic bacteria, remained stable throughout Phase 1 with values of 88 ± 4 and 68 ± 19 mgO_2 g VSS^{-1} d^{-1} at start-up and cycle #44, respectively. The specific activity on glucose, used as an estimation for the activity of acidogenic bacteria, increased fourfold, with 1093 ± 103 mgGle g VSS^{-1} d^{-1} at start-up compared to 4459 ± 316 mgGle g VSS^{-1} d^{-1} at the end of Phase 1 (Table 3). This suggests that the acidogenic population of the digester became predominant over time, while the methanogenic population in the biomass bed decreased. This would mean there was a replace-

Table 3
Biomass specific activities during Phase 1 of the SBR operation.

Cycle	Acetate (mg g VSS^{-1} d^{-1})	Hydrogen (mg g VSS^{-1} d^{-1})	Oxygen (mg g VSS^{-1} d^{-1})	Glucose (mg g VSS^{-1} d^{-1})
Start-up	148 ± 30	231 ± 57	88 ± 4	1093 ± 103
Day 14 – cycle #3	232 ± 40	404 ± 1	ND	ND
Day 36 – cycle #14	326 ± 60	291 ± 33	ND	ND
Day 85 – cycle #29	317 ± 6	59 ± 2	ND	ND
Day 127 – cycle #44	114 ± 8	65 ± 3	68 ± 19	4459 ± 316

ND: not determined.

ment of the methanogenic bacteria by acidogenic bacteria over time in the digester, the relative weight of the methanogens decreasing for a concomitant increase in acidogens bacteria, per unit of volatile solids. This population shift was possibly related to the faster growing rate of the acidogens and the fast-acidifying properties of the whey wastewater potentially generating high concentrations of VFA and inhibiting or at least destabilizing the process. While the specific activity on oxygen was, as expected, lower than reported by Gutiérrez et al. (2006) for an aerobic SBR (100–400 mgO_2 g VSS^{-1} d^{-1}), it is interesting to note that their calculated specific activity (3400–6900 mgCOD g VSS^{-1} d^{-1}) was not significantly different from the acidogenic activity of our sequential SBR.

3.4. Digester performance during Phase 2

Phase 2 of the experiment could be related to a pseudo steady-state operation of the digester since it consisted of 16 cycles of 48 h T_C each, with 26 h of anaerobic digestion, 16 h of aeration, 4 h of settling, 1 h for emptying and 1 h for filling. The OLR was also kept constant at 1.5 ± 0.1 gCOD L_{rx}^{-1} d^{-1}. The aeration was controlled at a dissolved oxygen concentration of 0.5 mg L^{-1} during the aeration step, which resulted in a higher oxygen input compared to the conditions applied during Phase 1. This was performed in an attempt to achieve residual COD in the same range as the one obtained during Phase 1 at 3 days T_C. The dissolved oxygen concentration used in this study was the same as the lowest dissolved oxygen concentration allowed during the operation of an aerobic SBR used for polishing whey wastewater after a first anaerobic SBR treatment (Li and Zhang, 2004). In their case, the organic load varied between 3 and 5 g VSS L^{-1} d^{-1} for 2.5–5 days HRT, and 89% COD removal was achieved.

There was a decrease in the pH value when operating during the anaerobic step, possibly because of the increase in VFAs concentration, specifically propionate (Table 4). However, the pH remained in an optimal zone during the incubation, and resumed at 7.42 ± 0.27 on average at the end of the cycles. After the anaerobic step, there was a COD removal of 72 ± 3% with residual sCOD of 1115 mg L^{-1}, which was higher than what was observed during Phase 1 at similar OLR and T_C (736 ± 390 mg L^{-1}). The whey wastewater degradation continued during the aerobic step with further reduction of the COD by around half (57 ± 10%), to 482 ± 115 mg L^{-1}. The total COD removal reached 88 ± 3% on average for the 16 cycles. These results were not statistically different than what was obtained during Phase 1 at the same T_C and OLR ($P = 0.117$). These results are comparable to the average values reported in the literature (Demirel et al., 2005) for similar conditions (OLR 0.5–2.5 gCOD L^{-1} d^{-1}; HRT 1–5 days; COD removal 80–90%).

The COD removal after each of the anaerobic and aerobic step of the cycles is shown in Fig. 3. A constant decrease in the COD removed after the anaerobic step was observed from cycle #1 to #6. This could be caused by the intensity of the aeration, at levels that could be resulting in an inhibition or even toxicity for the methanogens. The COD removal after the anaerobic step was maintained after cycle #6, with a slight overall increase over time. The shielding of the methanogens inside the anaerobic granules by the growth of the acidogens in the outer layer could be responsible for the stabilization of the anaerobic performance over time.

The VFA concentration was determined for each cycle after anaerobic and aeration steps (Table 4). Butyrate was rarely found in the effluent and remained below 10 mg L^{-1}. The acetate concentration was low at 79 ± 25 and 36 ± 19 mg L^{-1} after anaerobic and aerobic sequence on average. However, the propionate concentration remained high at 565 ± 59 and 245 ± 66 mg L^{-1} after anaerobic and aerobic steps. The VFAs were responsible for up to 92% of the residual sCOD left at the end of the cycle, indicating that the degradation of the whey wastewater was incomplete. This is in agreement with the work of Arbeli et al. (2006) who reported high propionate concentration during the anaerobic degradation of a dairy wastewater in a deep reservoir. The rate-limiting step of their process was the propionate oxidation and low temperature (15 °C) coupled to higher organic load was responsible for the propionate

Table 4
Operational parameters during Phase 2.

Parameters	In	An	Ae
pH	7.16 ± 0.30	6.95 ± 0.21	7.42 ± 0.27
Residual sCOD	4038 ± 236	1115 ± 127	482 ± 115
Acetate	188 ± 135	60 ± 27	37 ± 10
Propionate	243 ± 181	573 ± 48	223 ± 43
Butyrate	28 ± 58	<10	<10
VFA COD-eq	619 ± 449	925 ± 155	428 ± 160

In: initial; An: after anaerobic step; Ae: after aerobic step.

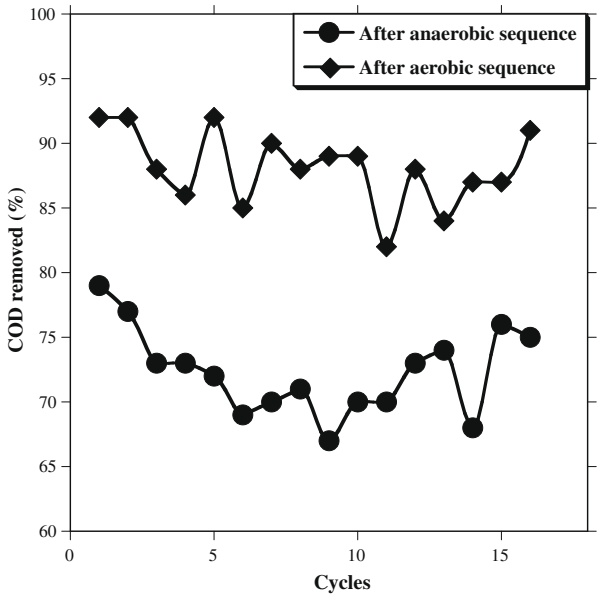

Fig. 3. COD degradation efficiencies after anaerobic and aerobic sequence during Phase 2 experiment.

accumulation in their system. In their case, propionate degradation resumed only when temperature was raised at 29 °C. High residual COD of 150–300 mg L^{-1} were also reported by McHugh et al. (2006), however it was presumed to consist of non-biodegradable material or soluble microbial product, since no significant amount of VFA were present in the effluent.

A close monitoring of the biogas production during the anaerobic step and sCOD removal during the aerobic step was performed during the last three cycles of Phase 2. An average of 356 ± 10 L of biogas was produced after the first 22 h of reaction time. Fig. 4a clearly shows that, even though there is a small inflexion point at hour 17, the anaerobic digestion of the whey wastewater is not completed at the end of the anaerobic step. Fig. 4b is showing the residual sCOD obtained from hourly grab samples. Again, the degradation of the whey wastewater is still going on linearly (R: 0.96–0.99) at the end of the cycle, clearly indicating the incomplete degradation of the whey wastewater.

The degradation of whey wastewater at 20 °C in an SBR digester would therefore require T_C of at least 3 days, as was shown during Phase 1 of the experiment, and increased aeration could not provide better and/or faster degradation of the wastewater, in order to reduce the duration of the cycle.

While the residual sCOD concentration was 466 ± 86 mg L^{-1} on average for those cycles, the BOD, total Kjeldahl nitrogen and total phosphorous concentration were 400 ± 113, 18.0 ± 4.0 and 13.3 ± 2.1 mg L^{-1}, respectively. Most of the residual sCOD was thus still biodegradable (VFA) at the end of the cycles, but nutrients were still present.

3.5. Biomass volume and specific activities during Phase 2

The biomass in the digester was evaluated at the beginning and end of the Phase 2 experiment. The biomass concentration increased in the digester over time, from 3.7 to 4.9 kg VSS, because of an increase in the suspended flocs quantity (visual estimate). The average specific OLR was around 0.13 gCOD g VSS^{-1} d^{-1}. The concentration of volatile solids was found to be significantly different in the biomass bed, compared to the suspended flocs, with 120 and 35 g VSS L^{-1}, respectively.

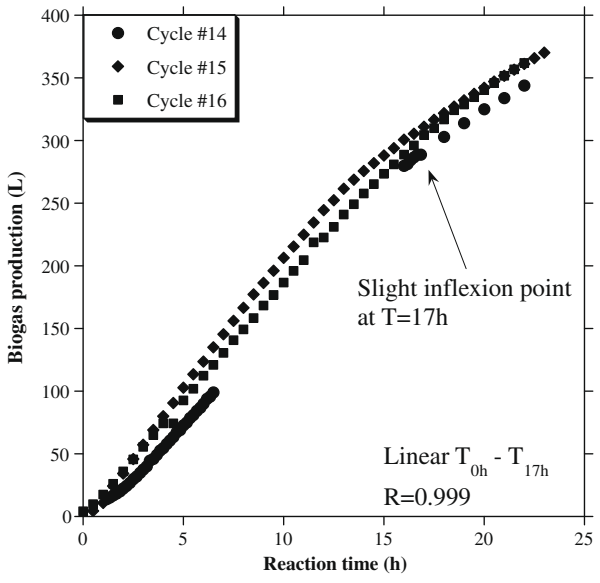

Fig. 4a. Biogas production during the anaerobic sequence for the last cycle of Phase 2 experiment. The percentage of methane was measured at hour 1, 3, 5, 16 and 22 for values of 7, 38, 41, 57 and 57.2%, respectively.

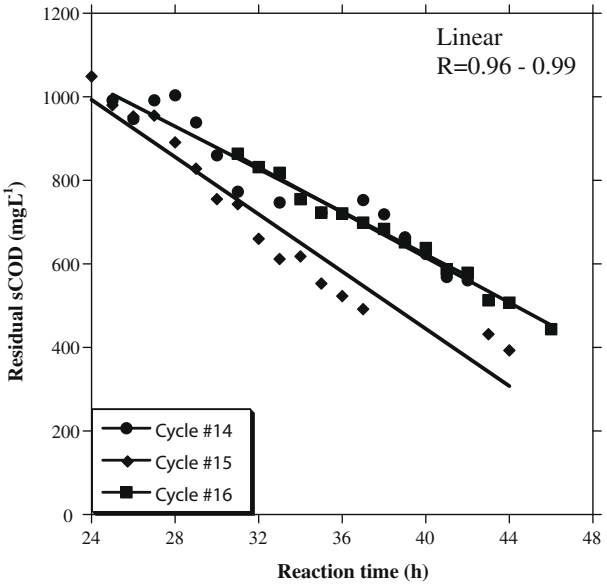

Fig. 4b. Residual sCOD during the aerobic sequence for the last cycle of Phase 2 experiment.

Table 5
Biomass specific activities during Phase 2 of the SBR operation.

Cycle	Acetate	Hydrogen	Oxygen	Glucose
Start-up	114 ± 8	65 ± 3	68 ± 19	4459 ± 316
Final				
Biomass bed (granules)	93 ± 7	57 ± 5	92 ± 20	2374 ± 42
Biomass flocs	0	29 ± 4	144 ± 12	6982 ± 545

All values in milligram substrate per gram VSS and per day.

The specific activity of the biomass was measured at the startup on the biomass bed composed of granules, and on the suspended biomass made of flocs, at the final cycle (Table 5). The methanogenic activities, already low at the beginning of the Phase 2 in the biomass bed, decreased slightly by 18% for the acetoclastic activity and 12% for the hydrogenotrophic activity. No acetoclastic activity was measured in the suspended flocs and the hydrogenotrophic activity was significantly reduced by 55%. The low methanogenic activity in the biomass bed, and the absence or very low methanogenic activity in the flocs, coupled to the relative lower weight of the biomass bed, resulted in a reduced amount of methanogens in the digester over time. The specific activity for the glucose showed a significant acidogenic population growth in suspended flocs, with 6982 ± 545 mgGle VS^{-1} d^{-1} while it decreased to 2374 ± 42 mgGle VSS^{-1} d^{-1} in the biomass bed. This is consistent with previous studies (Guiot et al., 1992). The oxygen activity increased slightly in the biomass bed and more than doubled in the suspended biomass. Hence, the coupling of an anaerobic and an aerobic steps in the SBR resulted in a compartmentalization of the biomass, with methanogenic biomass maintained in the bed and acidogenic biomass activity displaced from the biomass bed to a suspended floc.

4. Conclusions

A stable operation of sequential anaerobic and aerobic steps pilot-scale SBR digester was maintained for the biodegradation of a reconstituted whey wastewater during a period of 6 months. A total cycle time of at least 3 days was required to obtain satisfactory COD removal (97%) and residual sCOD (33 mg L^{-1}). The increase in

aeration during the aerobic step for Phase 2 of experimentation did not improve the overall performance of the SBR, with residual sCOD of 463 ± 122 mg L^{-1}, mainly composed of propionate, at T_C of 2 days. The methanogenic specific activities were low for most part of the experiment, while the acidogenic specific activities increased significantly over time. The biomass specific activities during Phase 2 showed a compartmentalization with methanogenic activities maintained in the biomas bed and most of the growth occurring under the form of suspended flocs which were mainly composed of an acidogenic population. The nature of the whey wastewater, rich in lactose, would probably favor such a rapid growth of acidogens.

The concept of coupling anaerobic and aerobic steps inside one digester still looks promising but T_C longer than 2 days are required to efficiently remove the biodegradable fraction of the whey wastewater, at 21 °C. Sequential anaerobic and aerobic degradation of the whey wastewater could be enhanced by improving the compartmentalization of the anaerobic and aerobic biomass inside the digester. The small-scale dairy farms and cheese producers are discharging as little as 200 m^3 of wastewaters per year. In this case, a full-scale process would only require a SBR digester of 2 m^3. Therefore, the results obtained during this study could be directly upscaled to the full-scale digester.

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