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Strategy to identify the causes and to solve a sludge granulation problem in methanogenic reactors: application to a full-scale plant treating cheese wastewater

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Abstract Granulation of biomass is at the basis of the operation of the most successful anaerobic systems (UASB, EGSB and IC reactors) applied worldwide for wastewater treatment. Despite of decades of studies of the biomass granulation process, it is still not fully understood and controlled. "Degranulation/lack of granulation" is a problem that occurs sometimes in anaerobic systems resulting often in heavy loss of biomass and poor treatment efficiencies or even complete reactor failure. Such a problem occurred in Mexico in two full-scale UASB reactors treating cheese wastewater. A close follow-up of the plant was performed to try to identify the factors responsible for the phenomenon. Basically, the list of possible causes to a granulation problem that were investigated can be classified amongst nutritional, i.e. related to wastewater composition (e.g. deficiency or excess of macronutrients or micronutrients, too high COD proportion due to proteins or volatile fatty acids, high ammonium, sulphate or fat concentrations), operational (excessive loading rate, sub- or over-optimal water upflow velocity) and structural (poor hydraulic design of the plant). Despite of an intensive search, the causes of the granulation problems could not be identified. The present case remains however an example of the strategy that must be followed to identify these causes and could be used as a guide for plant operators or consultants who are confronted with a similar situation independently of the type of wastewater. According to a large literature based on successful experiments at lab scale, an attempt to artificially granulate the industrial reactor biomass through the dosage of a cationic polymer was also tested but equally failed. Instead of promoting granulation, the dosage caused a heavy sludge flotation. This shows that the scaling of such a procedure from lab to real scale cannot be advised right away unless its operability at such a scale can be demonstrated.

Keywords Anaerobic · Cationic polymers · Cheese wastewater · Granulation · UASB reactor · Water upflow velocity · Trace metals

Abbreviations

COD	Chemical oxygen demand
D1	Digester 1
D2	Digester 2
DAF	Dissolved air flotation
$E_{Ag/AgCl}$	Redox potential according to Ag/AgCl reference electrode
EGSB	Expanded granular sludge bed
FOG	Fats, oil and grease
FOG_{COD}	FOG expressed as COD equivalent
FOG_{SLR}	FOG sludge loading rate
GLSS	Gas-liquid-solid separator
HRT	Hydraulic retention time
HT	Homogenisation (buffer) tank
IC	Internal circulation

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LCFA	Long-chain fatty acids
OLRs	Sludge organic loading rate
OLR_v	Volumetric organic loading rate
PW	Pumping well
SRT	Solid retention time
SVI	Sludge volumetric index
TSS	Total suspended solids
VFA	Volatile fatty acids
VSS	Volatile suspended solids
V_{up}	Water upflow (superficial) velocity
UASB	Upflow anaerobic sludge blanket

Introduction

Granulation of biomass is at the basis of the operation of the most successful anaerobic systems (UASB, EGSB and IC reactors) applied worldwide for wastewater treatment (van Lier et al. 2015). Despite of decades of studies of the biomass granulation process, it is still not fully understood and controlled (McHugh et al. 2003; Abbasi and Abbasi 2012). "Degranulation/lack of granulation" is a problem that occurs sometimes in this type of anaerobic systems resulting often in heavy loss of biomass and poor treatment efficiencies or even complete reactor failure (e.g. Clark 1988; Maat and Gorur 1990; Grootaerd et al. 1999; Ahn et al. 2001; McHugh et al. 2006; Borzacconi et al. 2008; Li et al. 2008; Sekiguchi et al. 2015).

The causes of granulation problems observed remain uncertain in most cases. Nevertheless, the information available in the literature suggests that they can be related to nutritional or non-nutritional factors such as

1. A deficiency or excess of some macronutrients (N, P, S) or micronutrients (Fe, Ni, Co, Mo, Ca, Al, Na, K, Se, W) that affect sludge growth, granule stability, or may cause their mineralisation—i.e. replacement of active biomass by inorganic precipitates (Guiot et al. 1988; Grotenhuis et al. 1991; Alphenaar et al. 1993; El Mamouni et al. 1995; Kim et al. 2004; Jeison et al. 2008; Speece 2008; Zandvoort et al. 2006, Mañas et al. 2012).
2. A high concentration of proteins, ammonium, phosphate, sulphate or sulphide (Vanderhaegen et al. 1992; Thaveesri et al. 1994; Kobayashi et al. 2015).
3. A conversion of substrates to volatile fatty acids higher than 40% of COD in the buffer tank since most VFA are poor growth inducers due to their low energetic content (Vanderhaegen et al. 1992).
4. A high concentration of fats (long-chain fatty acids or triglycerides) that adsorb to the biomass inducing its flotation and possibly granule disintegration (Amaral et al. 2004; Chipasa and Medrzycka 2006).
5. The presence of toxic compounds such as those brought by the disinfectants and cleansing agents which may induce cell lysis and deteriorate granule strength (Costa et al. 2007, 2013; Zitomer et al. 2007).
6. A wastewater liquid surface tension where granule formation is thermodynamically difficult if not impossible, or selects for granules with a hydrophobic external layer allowing biogas bubble adherence and leading to sludge washout (Thaveersi et al. 1995).
7. "Water and gas" superficial velocities within the reactors below or over the optimal ranges (e.g. 0.5–2 m/h for water) known to favour cell adhesion (i.e. the only way for cells to resist washout in these upflow systems), but also to avoid excessive erosion of the resulting microbial particles (Arcand et al. 1994; O'Flaherty et al. 1997; Bhunia and Ghangrekar 2008).

Degranulation was identified at the anaerobic wastewater treatment plant of a cheese factory near the city of Queretaro in Mexico. During the 33rd and 34th months of the reactor operation, a close inspection of the plant was performed in order to determine if some of the factors (1, 2, 3, 4 and 7) listed above were involved. In parallel to this investigation, an artificial granulation of the biomass was attempted by dosing the reactors with a high molecular weight cationic polymer. This endeavour was based on the excellent results of enhanced granulation obtained at lab scale by Cail and Barford as early as 1985 and reproduced since then with success regularly in the literature with different lab-scale anaerobic systems, wastewaters and polymers (e.g. El Mamouni et al. 1998; Wang et al. 2005; Ariyavongvivat et al. 2015). The results of these surveys are the object of the present paper.

Material and methods

Description of the anaerobic treatment plant

The cheese factory where the treatment plant is operating generates 130 to 172 m^3/d of wastewater resulting mainly from washing operations involving detergents (caustic soda or phosphoric acid based) and disinfectants (sodium hypochlorite, peracetic acid, hydrogen peroxide, iodine) and for a smaller part (~12 m^3/d) from the staff toilets and showers. Whey is normally commercialised. Occasionally however, when it acidifies and cannot be sold, it is also discharged into the sewers. In order to comply with discharge limits, the cheese factory constructed a wastewater treatment plant consisting of a physicochemical pre-treatment step based on dissolved air flotation to remove FOG, phosphate and suspended solids followed by a biological secondary step with two anaerobic reactors (D1 and D2) to remove dissolved COD (Fig. 1). In more detail, within this scheme, the process

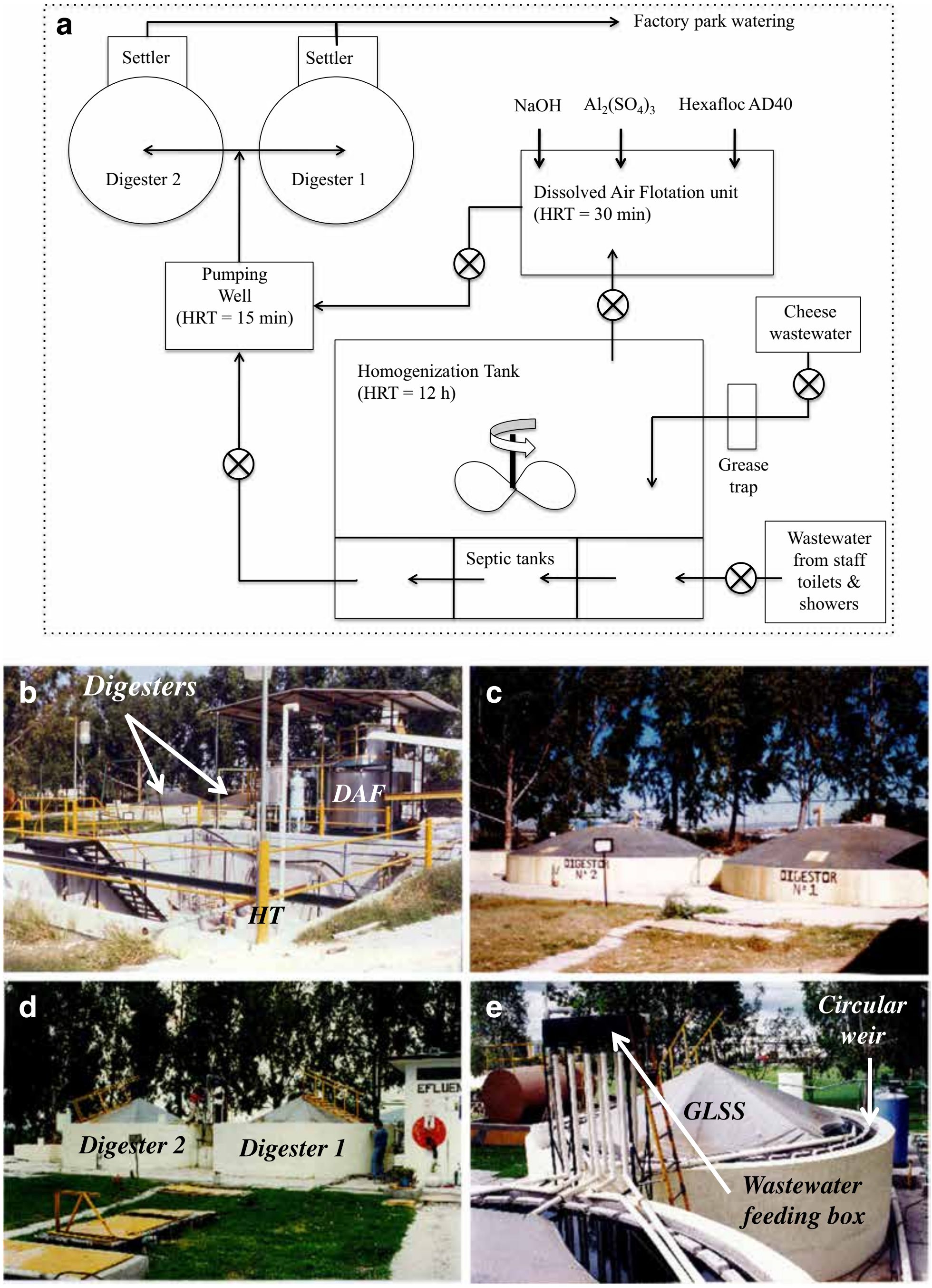

Fig. 1 **a** Block process flow diagram of the cheese factory wastewater treatment plant. **b** General view of the plant with the homogenisation tank in the *front*, the DAF system in the *back right side* and the two circular covered digesters before remodelling in the *back left side*. View of the digesters **c** before remodelling and **d** after remodelling. **e** Detailed view of one of the digesters and of the wastewater feeding system (distribution box and pipes) after remodelling

wastewater coming from the factory passed first by a grease trap to remove the easily separable FOG in order to avoid their accumulation within the feeding pipes, then by an agitated homogenisation tank with 12-h hydraulic retention time to buffer pH and COD variations and finally by a DAF where aluminium sulphate and an anionic polyacrylamide polymer (Hexafloc AD40 from Technics International) were added to flocculate the FOG and TSS. Caustic soda was also added at this step in order to maintain an optimum pH for the flocculation. The DAF-pre-treated effluent was directed towards a pumping well where it was mixed with the sanitary wastewater previously treated in septic tanks in order to hydrolyse faecal material. The flow rate coming from the pumping well was then divided equally between the two digesters. These digesters corresponded to identical pre-existing circular and covered tanks (Fig. 1c) with a conic base and a working volume of 60 m^3 each. They were both equipped with a four-exit submerged feeding box located at the bottom of the vessels in order to distribute the wastewater in an upflow mode as homogenously as possible within the sludge bed. At the start of the study, no gas-liquid-solid separator device was installed at the top of the reactors. Each of them had also only one single point exit of the treated water that was connected to a box intended to operate as settlers and retain part of the TSS carried with the water but without recycling to the digesters. The wastewater treated in this way was used for the watering of a plot of 5 ha, property of the company. Thanks to the homogenisation tank, the wastewater treatment plant could operate 365 days per year and 24 h a day.

Analytical procedures

All measurements were performed on 24-h composite water samples. COD (closed reflux colorimetric method, 5220D), TSS and VSS (methods 2540D and 2540E), pH (method 4500-H^+), temperature (method 2550), FOG (Soxhlet extraction, method 5520 D) and SO_4^{2-} (turbidimetric method 4500-SO_4^{2-} E) were determined according to Standard Methods (1995). Soluble COD was determined after filtration on a Whatman GF/A glass fibber membrane with porosity of 1.6 μm. The concentrations of total nitrogen (including nitrate and nitrite), total phosphorus and ortho-phosphate were obtained, respectively, with the Hach test N tube methods #10022, #10013 and #8048. Trace metals and cations were analysed with a PerkinElmer 500 IC plasma according to the instructions of the manufacturer. The samples for plasma analysis were prepared by microwave digestion according to EPA method 3015 for the water samples and EPA method 3051 for the sludge. Biogas CH_4 and VFA in wastewater were measured according to Celis-García et al. (2007). The particle size distribution of the reactors' sludge was determined by manual humid sieving as described by Laguna et al. (1999) and the sludge redox potential recorded as indicated by Macarie and Guyot (1995). The SVI was measured (method 2710 D) following Standard Methods (1995) except for lab-scale reactors for which the adaptation explained by Guyot et al. (1990) was applied.

Sludge methanogenic activity

The methanogenic activity of the sludge was determined with serum bottles of 100 mL filled under N_2 with a mixture of PW wastewater and sludge to have a COD/VSS ratio of 1 and a VSS concentration between 3 and 5 g/L. The bottles were incubated at 35 °C with shaking, and their gas phase was regularly sampled in order to follow the CH_4 concentration. The specific activity was determined from the slope of the linear phase of methane production divided by the amount of VSS in the bottle. Methane was later converted to COD considering that 15.625 mmol of CH_4 is produced per g of COD degraded.

Polymer addition

The cationic polymer Percol 763 (Allied Colloids Inc., USA) used for the artificial granulation attempt as well as its discontinuous dosage to the reactors whilst they are in operation were selected based on the work of El-Mamouni et al. (1998). Percol 763 presents the advantage of a flocculation capacity minimally affected by pH in the range 2 to 10. The dosage, necessary to obtain an optimal sludge flocculation, was pre-determined through jar tests and was found at 1 mg/g TSS. The absence of toxicity at this concentration for the reactors' biomass was also verified. The polymer was added seven times to the cheese factory reactor D2 in the form of a 0.25 or 0.5% (*w*/*v*) solution in tap water. The solution was dosed at different points of the plant and the dosage was adjusted from 0.12 to 1 mg polymer/g TSS (Table 1). Reactor D1 was initially used as control but received the final two doses of Percol 763. Originally, the dosage frequency was to be determined on the amount of TSS at the exit of the reactor. In reality, each polymer dosage was separated by 3 to 8 days.

Results and discussion

Full-scale reactor start-up, detection of the granulation problem, consequences and first tentative corrective actions

The two full-scale digesters were inoculated with the granular sludge of an anaerobic lagoon treating the wastewater of another cheese factory (Monroy et al. 1995). When wastewater was introduced, the size of the granules started to decrease.

Table 1 Frequency and conditions of dosage of Percol 763 to the industrial reactors

Day of operation	Place of dosage	Polymer solution concentration	Duration of dosage	Polymer dose	
				Reactor D1	Reactor D2
		% (*w*/*v*)		mg Percol 763/g sludge TSS	
974	Reactor top	0.25	3 min	–	0.24
978	Reactor top	0.5	3 min	–	0.23
986	Distribution box	0.25	7.5 h	–	1.00
992	Pumping well	0.25	7 h	–	0.27
998	Pumping well	0.25	1.25 h	–	0.10
1002	Pumping well	0.25	1.5 h	0.25	0.37
1008	Pumping well	0.25	1 h	0.28	0.12

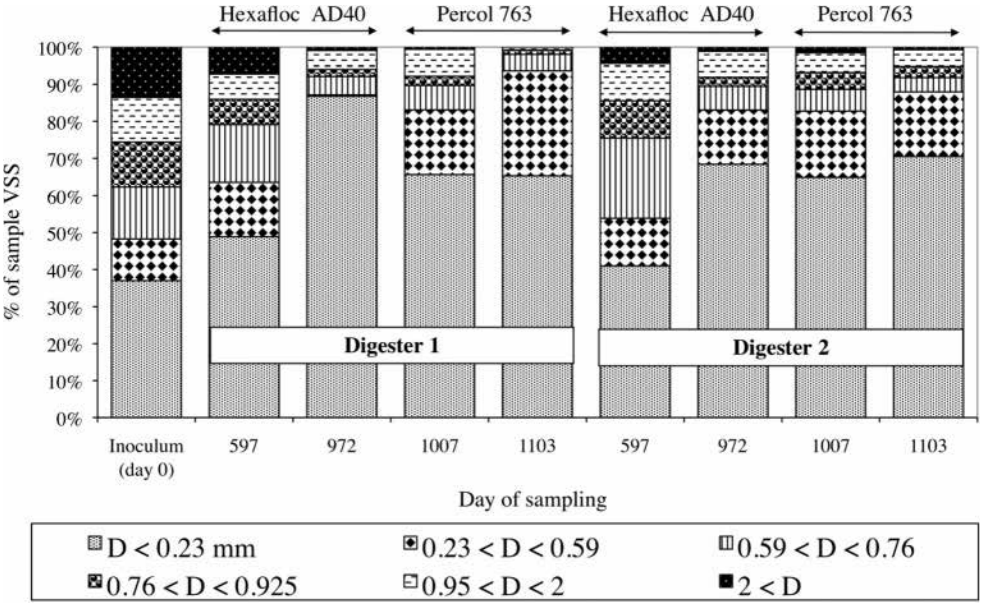

Fig. 2 Evolution of the granulometry of reactor D1 and D2 sludge over time and with the type of polymer used in DAF

The smaller particles were washed out of the reactors resulting in a heavy loss of biomass (effluent TSS > 200 mg/L). As a result, 19 months after the start-up, almost half of the initial sludge mass had been lost and 54 to 63% of the VSS still present in the digesters corresponded to particles smaller than 0.59 mm (Fig. 2; see values for day 597). The sludge organic loading rate had also reached values as high as 2–3 kg COD/kg VSS d whilst the sludge methanogenic specific activity was only 0.07–0.11 kg COD/kg VSS d. The SRT was less than 12–22 days. As a consequence, the reactors never reached the design performances (75% COD removal) and presented total COD removals of only 14–20% (22–24% for soluble COD) (Table 2; see data for reactor D1 and days 531 to 597). A reduction of the wastewater flow to reactor D2 during the same period in order to adjust its sludge organic loading rate to a more reasonable value of 1 kg COD/kg VSS d had no beneficial effect on COD removal (Table 2). Despite the overload imposed to the digesters, their mixed liquors always presented an adequate pH (6.6–6.7) and redox potential ($E_{\mathrm{Ag/AgCl}} = -389/-398$ mV).

In an attempt to increase the treatment plant performance, 1 year and 8 months after the start-up, the anaerobic reactors were transformed into UASB systems. The reactor modification included (1) the installation of a GLSS device to improve biomass retention (new reactor working volume 88.4 m^3), (2) the construction of a circular weir at the top of each reactor to collect homogeneously the treated water and eliminate eventual hydraulic short circuits and (3) the replacement of the submerged wastewater distribution system by an external distribution box, from which, five pipes were directed to the bottom of each sludge bed (1 inlet/5.6 m^2) to ensure a better contact between the microorganisms and the wastewater (Fig. 1e). Once the transformations were completed 3 months later, the reactor biomass content was also increased to 30 m^3 through the addition of fresh sludge from the same source as the original inoculum. The restart-up strategy consisted in adjusting the digesters' feeding rate to their biomass real treatment capacity. With these modifications, the reactor performances improved drastically. On day 972 (2 years and 9 months after the first start-up), they removed more than 74% and 87% of the total and soluble COD, respectively (Table 2). However, until this date, the flow rate that they accepted was limited to 14% of the flow rate reaching the plant (2 L/s), and any increase of this value was immediately translated into an important biomass washout. Additionally, the sludge granulometry remained poor with 83 to 87% of the sludge VSS corresponding to sludge particles smaller than 0.59 mm of which at least 68% were smaller than 0.23 mm (Fig. 2; see day 972). It is when possible causes for the degranulation/lack of granulation started to be investigated.

Search of nutritional factors: characterisation of wastewater composition

COD/N/P/S ratio

The characterisation of the wastewater at different points of the plant (Table 3) showed that the DAF removed 71.9 ± 19.8% of the FOG, 76.7 ± 10.4% of the TSS, 28.2 ± 16.7% of total nitrogen, 69 ± 9.5% of total phosphorus and 80% of the particulate COD whilst soluble COD removal was almost negligible (less than 10%). The concentrations remaining in the wastewater after mixing with the sanitary flow in PW resulted in a COD/N/P/S ratio of 100/2.3/0.95/6.58, which indicated that nitrogen, phosphorus and sulphur were, respectively, 1.6, 3 and 87 times

Table 2 Operating conditions and performances of the industrial reactors through days 531 to 1023

Parameters	Design values	Days of operation after first start-up			
		531–597	972	1008	1023
Pumping well					
COD total (g/L)	–	2.78±0.59	2.788	1.731	1.538
COD soluble (g/L)	–	2.38±0.55	2.374	1.355	1.200
TSS (g/L)	–	0.31±0.18	0.27	0.17	nd
FOG (g/L)	–	nd	0.359	0.156	0.156
Reactor D1					
Flow rate to the reactor (L/s)	0.7	1.02±0.12	0.16	0.31	0.5
Sludge mass (kg TSS/reactor)	–	238.41±143.9	569.07	535.27	1561.72
Sludge [VSS]/[TSS] (%)	–	46.72±16.73	49.34	51.82	46.75
OLR$_v$ (kg COD/m^3 d)	2	4.13±1.23	0.44	0.52	0.75
OLRs (kg COD/kg VSS d)	0.6	2.99±1.21	0.137	0.167	0.091
FOG$_{SLR}$ (g FOG$_{COD}$/g VSS d)	–	nd	0.048	0.041	0.025
HRT (days)	1	0.69±0.06	6.4	3.3	2.0
SRT (days)	90	12.03±9.72	147.0	85.0	271.8
V_{up} (m/h)	0.08	0.128±0.015	0.018	0.036	0.058
Total COD removal (%)	75	14.89±12.31	85.30	69.02	nd
Soluble COD removal (%)	80	22.93±18.40	93	76	97
TSS out (mg/L)	70	225±20	280	235	133
Reactor D2					
Flow rate to the reactor (L/s)	0.7	0.58±0.20	0.12	0.3	0.51
Sludge mass (kg TSS/reactor)	–	416.4±216.93	898.60	1267.88	1855.24
Sludge [VSS]/[TSS] (%)	–	36.50±14.86	50.92	51.49	48.16
OLR$_v$ (kg COD/m^3 d)	2	2.33±1.06	0.33	0.51	0.77
OLRs (kg COD/kg VSS d)	0.6	1.01±0.21	0.06	0.07	0.08
FOG$_{SLR}$ (g FOG$_{COD}$/g VSS d)	–	nd	0.022	0.017	0.021
HRT (days)	1	1.08±0.11	8.5	3.4	2.0
SRT (days)	90	38.93±29.88	234.2	123.8	206.4
V_{up} (m/h)	0.08	0.07±0.03	0.014	0.035	0.059
Total COD removal (%)	75	20.16±16.32	74.46	67.67	nd
Soluble COD removal (%)	80	24.01±19.05	87	81	98
TSS out (mg/L)	70	213±153	370	395	204

Values after ± correspond to standard deviation

nd not determined

in excess compared to the minimum ratio (100/1.43/0.28/0.075) usually recommended for biomass growth and so granule formation in anaerobic digestion.

Concentration of proteins, ammonium, phosphate, sulphate and sulphide

The forms under which nitrogen was present in the wastewater were not investigated. Nitrogen was probably present incorporated in proteins or as free ammonium or both since, unlike most other cheese plants, nitric acid cleaning agents were not used here (Danalewich et al. 1998). As mentioned previously, both ammonium and proteins are known to negatively affect the formation and growth of granular sludge pellets (Thaveersi et al. 1994). This occurs however at ammonium concentrations over 1167 mg N-NH$_4$/L (pH > 7.4) or when the proteins are at a concentration which cause a COD/N ratio higher than 100/3 (Thaveersi et al. 1994), two conditions that were never reached at the level of PW (Table 3).

Most of the SO$_4^{2-}$ (72.8%) detected in the wastewater was introduced through the dosage of 640 mg Al$_2$(SO$_4$)$_3$/L to the DAF. The resulting COD/SO$_4^{2-}$ ratio of 5 in the pumping well indicated that sulphate reduction should be important in the reactors. This was confirmed by a substantial decrease (80 to

Table 3 Wastewater composition at different points of the cheese factory treatment plant from day 973 to 1033

Sampling point (exit)	*T* (°C)	pH	FOG (mg/L)	TSS (mg/L)	COD (mg O_2/L)		N (mg N/L)	Phosphorus (mg P/L)		SO_4^{2-} (mg/L)	VFA (mg O_2/L)
					Total	Soluble	Total	Total	Reactive		
HT	27.8 ± 2.1	5.4 ± 0.4	652.2 ± 431.2	837.1 ± 525.8	3275.5 ± 827.2	1766.3 ± 655.8	67.6 ± 17.3	55.8 ± 9.9	49.7 ± 8.5	36.0 ± 53.3	–
DAF	27.5 ± 2.1	6.5 ± 0.6	–	154.2 ± 71.2	1752.2 ± 498.2	1605.7 ± 584.1	47.8 ± 13.3	17.5 ± 6.3	12.3 ± 4.3	269.4 ± 98.8	–
PW	*27.4 ± 1.9*	*6.6 ± 0.5*	*156.1 ± 149.8*	*183.8 ± 58.6*	*1874.3 ± 356.7*	*1575.7 ± 449.0*	*42.7 ± 19.3*	*17.7 ± 6.2*	*12.8 ± 4.6*	*369.8 ± 99.7*	*272.4 ± 166.0*
D1	23.1 ± 1.4	7.2 ± 0.2	–	186.9 ± 59.1	393.8 ± 101.2	137.3 ± 113.6	60.4 ± 27.2	16.9 ± 7.7	14.7 ± 2.2	67.3 ± 100.9	235.4 ± 101.9
D2	23.2 ± 1.4	7.3 ± 0.1	–	230.4 ± 98.1	451.8 ± 111.2	183.3 ± 128.3	55.0 ± 11.5	19.4 ± 5.4	14.7 ± 2.9	20.4 ± 31.0	223.9 ± 151.1

VFA are expressed in COD equivalent; values after ± correspond to standard deviation

T temperature, *N* nitrogen, *total phosphorus* = organic + mineral phosphorus, *reactive phosphorus* = $H_3PO_4 + H_2PO_4^- + HPO_4^{2-} + PO_4^{3-}$

100%) of sulphate concentration in their effluents (Table 3) and visually by the black colour of their biomass. In addition, sulphate reducers growing on lactate and some amino acids could be isolated from their sludge (Hernandez-Eugenio et al. 2000). Irrespective of the point of sampling in the treatment plant, 70 to 86% of the total phosphorus (72% in PW) corresponded to ortho-phosphate (Table 3). This was logical since 73% of the phosphorus detected in the raw wastewater could be attributed to the phosphoric acid-based detergents used to clean the pasteurisation and ultrafiltration equipments of the factory.

An excess of reactive phosphorus and SO_4^{2-} could be detrimental for sludge granulation. This is related to the capacity of PO_4^{3-}, HPO_4^{2-} and S^{2-} (product of sulphate reduction) to form poorly soluble complexes (solubility products between 10^{-7} and 10^{-35}) with several micronutrients indispensable for the growth of methanogens (Al, Co, Fe, Ni) or the stability of sludge aggregates (Ca^{2+}) and which will not be or will be less accessible to the microorganisms under a precipitated form. Theoretical calculations using expected concentrations of PO_4^{3-}, HPO_4^{2-} and S^{2-} in the anaerobic reactors and the solubility products (Ksp) of the different predicted complexes[1] suggested that the concentrations of Ca^{2+}, Co^{2+}, Fe^{2+} and Ni^{2+} in the mixed liquors of the reactors could be below the recommended levels (Table 4). The direct analysis of these elements showed that in fact they were all present in adequate amounts in the reactors' mixed liquors (see concentration at the reactor exit) except Co and Mo which could not be detected (Table 4). Co and Mo were also not detected in the sludge of reactor D1 whilst Mo was found in the sludge of reactor D2 but in very low amounts (Table 4).

An experiment was then set up to test further this apparent deficiency. It consisted to determine the methanogenic activity of reactor D2 sludge in presence of Co and Mo added separately or together at a concentration of 0.25 or 0.5 mg/L. No difference in the rate of methane production was observed compared to the activity determined in absence of added metals (Fig. 3). A similar result was obtained when acetate was used as carbon source instead of wastewater (data not shown). This suggested that, although undetectable with the analytical equipment used, the levels of Co and Mo in the wastewater were sufficient for not interfering with the methanogenic capabilities of the sludge.

Beside the capacity of S^{2-} to form insoluble complexes with trace metals, other product of sulphate reduction, soluble free H_2S, may equally affect the granulation process since it is known to inhibit the activity of the methanogenic *Archaea* and acetogenic *Bacteria* (Rinzema and Lettinga 1988). The concentrations of soluble-free H_2S in the digesters which could be calculated[2] accounting for (1) a complete reduction of the

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[1] $AlPO_4$, Al_2S_3, $Ca_3(PO_4)_2$, $CaHPO_4$, $Co_3(PO_4)_2$, $CoHPO_4$, CoS, FeS, $Ni_3(PO_4)_2$, NiS

[2] $[H_2S_{soluble}] = [S_{total\ soluble\ sulphide\ in\ reactors}]/[1 + 10^{[pH - pKa'1]} + 10^{[2 \times pH - pKa'2 - pKa'1]}]$. For the calculation of total soluble sulphide and the values of pH, pKa'_1 and pKa'_2, see Tables 3 and 4.

SO_4^{2-} present in PW, (2) the stripping due to biogas production estimated from COD removal and (3) the pH of their mixed liquors were of 42 and 36.9 mg H_2S-S/L for D1 and D2, respectively. Such H_2S levels may reduce the methanogenic acetoclastic activity by 20–30% (Rinzema and Lettinga 1988). Nevertheless, no negative effects on granulation build-up have been observed with sulphide concentrations up to 600 mg/L (Vanderhaegen et al. 1992). Even if the products of sulphate reduction (S^{2-}, H_2S) have no impact on sludge granulation, the fact that sulphate-reducing bacteria may represent a big part of the microbiota could affect granular stability since the ability of these bacteria to flocculate seems very poor (Visser et al. 1993). Fortunately, for a COD/SO_4^{2-} ratio between 5 and 10, the impact on sludge granulation appears to be limited to a 10–20% reduction of the granule growth yield compared to granules grown in the same conditions but in absence of sulphate (Vanderhaegen et al. 1992) which should then be the case here.

Na and K contents

Due to the use of brines for cheese processing, the NaCl and the other salts added could negatively affect sludge growth as members of the microbiota have specific halotolerance levels, outside of which the salts become toxic or growth-arresting. Levels of sodium and potassium were followed in parallel with trace metals and calcium. Their concentrations in PW (Table 4) were at levels much lower than those known to cause 10% inhibition of the acetoclastic methanogenic activity (Kugelman and Chin 1971; Rinzema et al. 1988). Potassium was at a concentration even close to the value (0.01 M or 390 mg/L) for which it gives an optimum antagonistic effect to the toxicity of Na^+ (Kugelman and Chin 1971). An increase of the ionic strength, related to the presence of these cations, could also negatively impact the physical integrity of the granules. However, it has been reported that NaCl concentrations of 125 mM (2.36-fold higher than at the digester entrance) do not affect the resistance of methanogenic granules to abrasion (Pereboom 1997).

VFA content

It was found that VFA represented 15.3 ± 11.15% of total COD and 19.7 ± 16.9% of the soluble one at the level of PW (Table 3). With the analytical conditions used, only acetic, propionic and butyric acid could be detected. They represented, respectively, 47.6 ± 6, 43.4 ± 7.6 and 9.1 ± 4.2% of the VFA-COD. According to previous works on acidogenesis of dairy effluents (e.g. Yu and Fang 2001), it is probable however that other compounds such as lactic acid and alcohols' (methanol, ethanol, others) were also produced in the buffer tank taking into account its pH (Table 3) and residence time (12 h). Further evolution of the wastewater in the DAF and PW was not expected due to the very short hydraulic retention time here (30 and 12 min, respectively). Lactic acid and alcohols are high-energy substrates[3] and as so are good carbon sources for granulation. This means that the COD of the detected VFA, which on the contrary are poor inducers of granulation[3], is sufficient to conclude that the level of acidification in the buffer tank was within the recommended range to avoid granulation problems (Lettinga and Hulshoff Pol 1991).

Fats, oil and grease content

Some 156.06 ± 149.82 mg/L of FOG was found at the entrance of the digesters (Table 3). Most of these corresponded to milk fat not removed by the DAF and were probably in the form of triglycerides and free long-chain fatty acids resulting from the expected rapid hydrolysis of the triglycerides in the homogenisation tank (Hwu 1997). The composition of the LCFA in PW was not determined, but could be approximated with that of milk which contains in % (*w*/*w*) of total LCFA (Hwu 1997), 7% lauric (2.72), 6% myristic (2.807), 21% palmitic (2.875), 6% stearic (3.104), 2% palmitoleic (2.834), 39% oleic (2.894) and 13% linoleic acids (2.82) (values in parenthesis correspond to the theoretical COD of each LCFA in g O_2/g). Since the theoretical COD of the LCFA and of their corresponding triglycerides is very similar, it is possible to estimate that 1 g of milk LCFA or triglycerides or mixture of them generates a theoretical COD of 2.7 g O_2 ($=\sum[(\mathrm{LCFA}_i\ \mathrm{COD}_{\mathrm{theoretical}}) \times (\%\ \text{of}\ \mathrm{LCFA}_i\ \text{in LCFA milk})]$). This means that 156.06 ± 149.82 mg/L of FOG should represent some 421.4 ± 404.5 mg COD/L. It results from this value that the FOG sludge loading rates supported by the digesters (Table 2) were well below the rate (0.09 g LCFA-COD/g VSS d) over which sludge flotation has been reported to start (Hwu et al. 1998). Moreover, this should remain true even if the digesters received all the wastewater flow from the cheese factory (1 L/s or 86.4 m^3/d each). As a consequence, the levels of FOG are presumably unrelated to the observed granulation problems. Nevertheless, this cannot be definitively excluded as an almost complete washout of granular biomass has been reported in the literature (Maat and Gorur 1990) for a full-scale UASB reactor treating at a similar FOG sludge loading rate (0.028 g FOG/g TSS d, estimated from given biomass inventory), the wastewater of a baking company containing also after pre-treatment by DAF a similar FOG concentration (136 mg/L). In this case, biomass loss could be clearly associated to FOG and solved by an improvement of FOG removal below 50 mg/L at the level of the DAF unit. It must be noted however that the reactor described in this study was operated

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[3] The Gibbs free energy of their conversion to CH_4 and CO_2 is of −1.145 kJ/g COD for lactate and higher than −0.9 kJ/g COD for the alcohols, while it is only of 0.55 and 0.56 kJ/g COD for propionic and acetic acids (see Vanderhaegen et al. 1992).

Table 4 Concentration of some trace metals and cations at different points of the plant (in mg/L) and in the sludge of the reactors (in mg/g TSS) and comparison to the expected solubility in the reactors' mixed liquor calculated from solubility products (Ksp) but also to the optimum concentrations reported for growth of methanogenic *Archaea* or granule formation (both in mg/L)

Metal	Water sampling points (exit)					Reactor sludge		Maximum solubility for complexes with		Optimum concentration for growth or granulation
	HT	DAF	PW	D1	D2	D1	D2	$PO4^{3-}/HPO_4^{2-}$	S^{2-}	
Al	1.0	1.2	1.0	1.1	1	11.29	6.7	$8.23\ 10^{-6}$	$> 10^{13}$	NA
Ca	125	125	109	125	94	46.88	39.12	8.5-145	NA	80 - 200[a]
Co	< 0.006[d]					< 0.00096[d]		0.1	$2\ 10^{-12}$-$6\ 10^{-8}$	0.00059 - 0.059[b]
Fe	0.02	0	0.2	0.1	0.3	11.82	8.35	NA	$9\ 10^{-6}$-$8\ 10^{-5}$	0.28 - 50.4[b]
K	156	188	188	266	484	15.62	31.25	NA	NA	< 3510[c]
Mo	< 0.0079[d]					$< 10^{-3}$[d]	0.00125	NA	NA	0.00096 - 0.048[b]
Na	813	1125	1219	1141	1000	39.12	70.37	NA	NA	< 4600[c]
Ni	6.8	10	4.5	4	5.6	0.5	0.7	2.87	$2\ 10^{-13}$-$4\ 10^{-6}$	0.0059 - 5[b]

The maximum solubilities were calculated at the pH of the reactors (Table 3) using the following equations:

$[\text{Cation}] = (\text{Ksp}/[PO_4^{3-}]^2)^{1/3}$ for $Ca_3(PO_4)_2$, $Co_3(PO_4)_2$, $Ni_3(PO_4)_2$; $[\text{Cation}] = \text{Ksp}/[PO_4^{3-}]$ for $AlPO_4$; $[\text{Cation}] = \text{Ksp}/[HPO_4^{2-}]$ for $CaHPO_4$, $CoHPO_4$; $[\text{Cation}] = (\text{Ksp}/[S^{2-}]^3)^{1/2}$ for Al_2S_3; $[\text{Cation}] = \text{Ksp}/[S^{2-}]$ for CoS, FeS, NiS

The concentrations of PO_4^{3-}, HPO_4^{2-} and S^{2-} in the reactors were calculated with the following formulas:

$[PO_4^{3-}] = [P_{\text{reactive in PW}}]/(1 + 10^{(\text{pKa1} + \text{pKa2} + \text{pKa3} - 3\text{ pH})} + 10^{(\text{pKa2} + \text{pKa3} - 2\text{ pH})} + 10^{(\text{pKa3} - \text{pH})})$; $[HPO_4^{2-}] = [PO_4^{3-}]/10^{(\text{pH} - \text{pKa3})}$

$[S^{2-}] = [S_{\text{total soluble sulphide in reactors}}]/(1 + 10^{(\text{pKa1}' + \text{pKa2}' - 2\text{ pH})} + 10^{(\text{pKa2}' - \text{pH})})$

The concentration of $S_{\text{total soluble sulphide in reactors}}$ was determined with the following formula adapted from Callander and Barford (1983, see Eq. 13 of the paper), considering that the only sulphide source was SO_4^{2-} and that 100% were reduced to sulphide in the reactors, which was true most of the time. Biogas production was not measured and was estimated from the average percent removal (E%COD) of soluble COD (CODs) in the digesters and the fact that 0.35 L of CH_4 are formed per g of COD removed and that biogas usually contains 60% CH_4 (*v*/*v*). Alfa in this formula corresponds to Henry's law constant for the equilibrium between gaseous and soluble H_2S

$[S_{\text{total soluble sulphide in reactors}}] = [SO_4^{2-}{}_{\text{ in PW}}]/(1 + (([\text{CODs}_{\text{ in PW}}] \times (\text{E\%COD}_{\text{in D1 or D2}}/100) \times 0.35)/(\alpha \times (1 + 10^{-\text{pKa1}'}/10^{-\text{pH}}) \times 0.6)))$

The Ksp for the calculations were taken from Callander and Barford (1983) and Dean (1992): 6.3×10^{-19} for $AlPO_4$, 2×10^{-29} and 10^{-25} for $Ca_3(PO_4)_2$, 10^{-7} for $CaHPO_4$, 2×10^{-35} for $Co_3(PO_4)_2$, 2×10^{-7} for $CoHPO_4$, 5×10^{-31} for $Ni_3(PO_4)_2$, 2×10^{-7} for Al_2S_3, 2×10^{-25} and 4×10^{-21} for CoS, 10^{-18} and 6×10^{-18} for FeS, $2 \times 10^{-26}/1 \times 10^{-24}/2 \times 10^{-21}$ and 3.2×10^{-19} for NiS

pKa values (25 °C) and α (35 °C) were from Callander and Barford (1983): $\text{pKa}_1 = \text{pKa}\ (H_3PO_4/H_2PO_4^-) = 2$, $\text{pKa}_2 = \text{pKa}\ (H_2PO_4^-/HPO_4^{2-}) = 7.3$, $\text{pKa}_3 = \text{pKa}\ (HPO_4^{2-}/PO_4^{3-}) = 12.3$, $\text{pKa}_1' = \text{pKa}\ (H_2S/HS^-) = 7$, $\text{pKa}_2' = \text{pKa}\ (HS^-/S^{2-}) = 12.9$, $\alpha = 1.83$

NA not applicable

[a] Optimum concentrations were from El Mamouni et al. (1995) (granulation)

[b] Optimum concentrations were from Speece (2008)

[c] Optimum concentrations were from Kugelman and Chin (1971)

[d] Detection limit

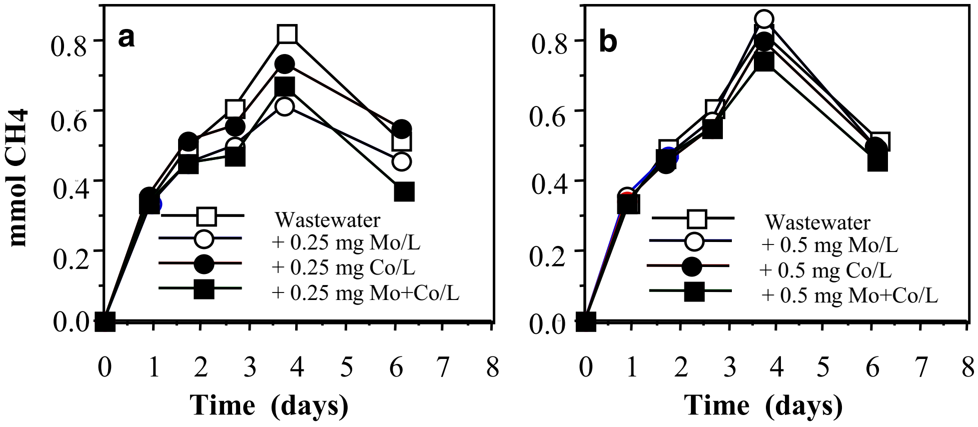

Fig. 3 Evolution of the methane production of reactor D2 sludge on PW wastewater at a COD/VSS ratio of 1 in presence and absence of 0.25 (**a**) or 0.5 mg/L (**b**) of Co and Mo or both of them

at much higher volumetric loading rate (6.8 kg COD/m^3 d) and lower HRT (7 h) than the present cheese factory reactors resulting in much higher water (0.65 m/h) and biogas (0.23 m/h) superficial velocities which would drastically increase the potential for sludge flotation and washout.

Polymer addition

The first two additions of Percol 763 resulted in the formation of a 60-cm layer of floating biomass at the surface of reactor D2 giving a temporary important loss of solids. This occurred despite the use of a Percol dosage (Table 1) below the optimum value identified during jar test. The problem was probably related to the trapping of biogas in the flocs and could be magnified by the place (top of the reactor) but also the duration (3 min) of the addition. As a consequence, it was decided to add the third dose very slowly and through the reactor distribution box in order to better disperse the polymer in the sludge bed (Table 1). In spite of these modifications, the same phenomena occurred but this time the thickness of floating biomass was less (15 cm). The reduction of flotation was particularly significant as on this occasion the amount of Percol had been increased by a factor of 5 to reach the theoretical optimum flocculation level. Based on these results, the next four additions of Percol 763 were performed in PW, and the doses were reduced to 0.1–0.37 mg Percol/g TSS. The duration of the dosage was maintained between 1 and 7 h (Table 1). In this way, sludge flotation could be controlled and reduced to a minimum. In order to keep reactor D1 as a control, its operation was stopped

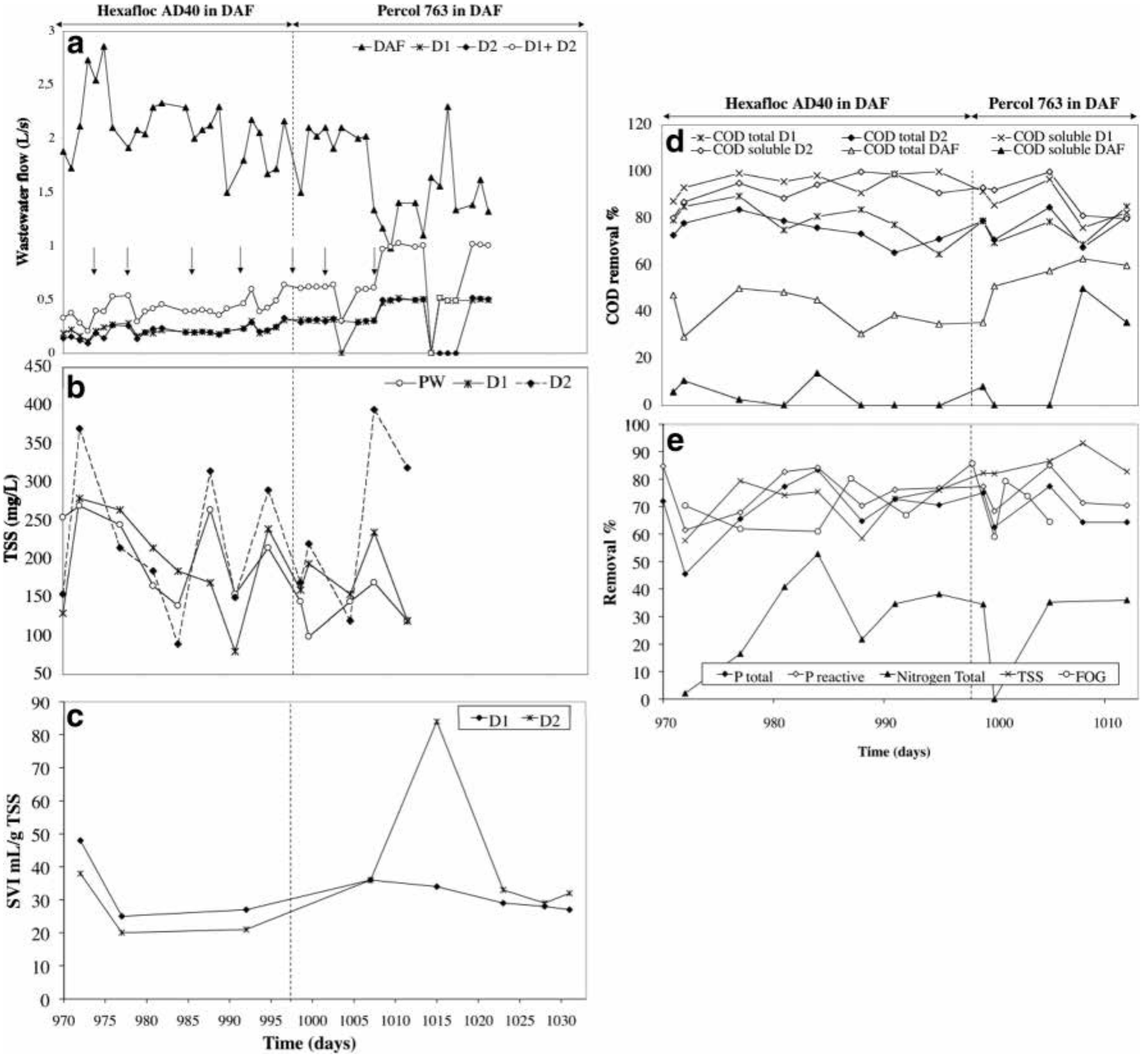

Fig. 4 Effect of Percol 763 addition on **a** the flow rate which can be fed to the reactors, **b** their TSS loss, **c** the SVI of their biomass, **d** their COD removal efficiencies together with that of the DAF and **e** the DAF phosphorus, nitrogen, TSS and FOG removal efficiencies. The *arrows* in **a** corresponds to the addition of Percol directly to D2 and later D1

during the fourth and fifth Percol additions. Nevertheless, the last two doses were distributed to both reactors (Table 1).

As shown in Fig. 4b, despite the biomass flotation described above, the addition of Percol 763 did not globally improve or worsen the loss of TSS by reactor D2 compared to reactor D1. Just, before the seventh addition of Percol (day 1007), the measurement of the particle size distribution of the sludge of both reactors showed that no granulation had been obtained (Fig. 2). Over 60% of D2 sludge VSS still corresponded to particles smaller than 0.23 mm as at the beginning of the plant follow-up 35 days before (day 972). The apparent improvement of D1 sludge granulometry on the same day was probably a sampling artefact. In a similar manner, over the same period, no improvement of the SVI of D2 sludge compared to D1 sludge was observed (Fig. 4c).

Following visual inspection, it appeared that the flocs obtained with Percol 763 disaggregated 2 or 3 days after being formed. This is common and usually due to the loss of stability of the polymers over time. Nevertheless, in the present case, this phenomenon was apparently the result of an antagonistic effect due to a fraction of the high molecular weight anionic polymer (Hexafloc AD40) used in the DAF unit which did not react locally and was lost to the wastewater. Qualitative experiments clearly demonstrated that D2 sludge flocculated with Percol 763 (0.5 mg/g TSS) could not maintain its structure after addition of Hexafloc AD40 (0.5 mg/g TSS).

As Percol 763 is also recommended for phosphorus removal in combination with alum, on day 998, it was decided to use it instead of Hexafloc AD40 in the DAF operation. Quickly after this change, it was possible to increase the flow rate of wastewater to the reactors without observing any biomass washout or deterioration of their COD removal efficiencies (Fig. 4a, b, d). The DAF COD, FOG, TSS, phosphorus and nitrogen removal efficiencies remained unaffected by the change of polymer (Fig. 4d, e). Two more additions of Percol 763 to PW were still done after day 998 (Table 1). One week after the second addition, D2 sludge became suddenly bulkier with a SVI of 80 mL/g TSS (normal range 20–30 mL/g; Fig. 4c). No clear explanation to this phenomenon was identified but the decision was taken to stop the direct dosage of Percol to the digesters.

At the end of the period of study (day 1033), both reactors were treating 79.1 ± 12.4% of the total wastewater flow produced by the factory. The activities of D1 and D2 sludge measured on day 1007 (0.601 ± 0.03 and 0.417 ± 0.032 g COD/g VSS d for reactors D1 and D2, respectively) showed nevertheless that the reactors had the capacity to treat the daily COD load remaining after the physicochemical pre-treatment. In agreement with this, according to the cheese factory staff, the reactors were finally able to treat the nominal flow rate. The last granulometry determination performed on day 1103 indicated however that 3 months after the switch of polymers in the DAF, the reactors' biomass was still flocculent (Fig. 2). This was

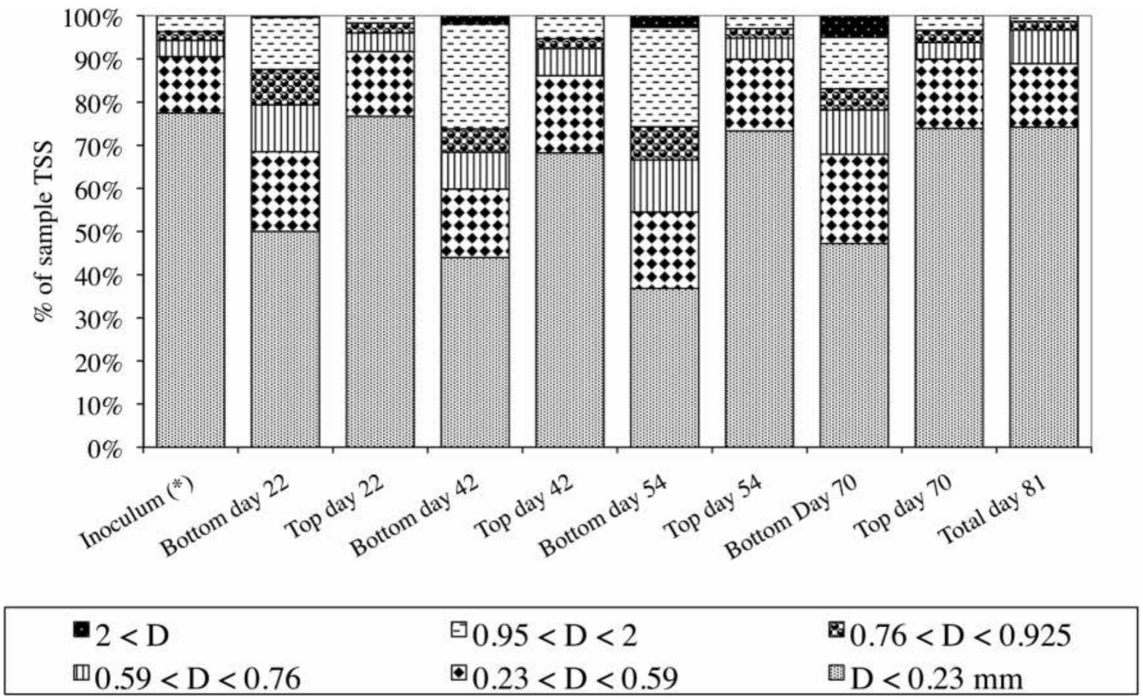

Fig. 5 Evolution of the sludge granulometry of the lab-scale reactor operated at 0.5 m/h (*asterisk* indicates the sludge sampled on reactor D2 on day 1344)

unchanged after 1 year of operation using these new conditions (see values for the inoculum in Fig. 5).

Test of the effect of water upflow velocity on granulation at lab scale

The V_{up} of the cheese factory reactors (Table 2) being well below the range (0.5–2 m/h) recommended for granulation (Arcand et al. 1994), and this parameter being relatively easy to manipulate at full-scale by recycling part of the treated water, two lab-scale UASB reactors of 2 L were set up in order to evaluate if an action on this parameter could induce granulation. The test was not done at full scale because no recirculation facilities had been planned in the original design of the reactors and because no pumps of the required capacity were available on site. Logically, their purchase would be only considered in case of success of the experiment. The two lab-scale reactors were inoculated with the sludge of reactor D2 and fed with PW wastewater. Before start-up, the ratio "sludge volume/reactor working volume" was of 50% as in the full-scale reactors. The HRT (2 days) and the OLRs (0.05–0.15 kg COD/kg VSS d) applied to both reactors throughout the experiment were identical to those of the real plant, but one was operated at a V_{up} of 0.5 m/h and the other at the V_{up} of the full-scale reactors (0.06 m/h on day 1023). Such V_{up} were achieved by water recycling. A V_{up} higher than 0.5 m/h could not be tested since this resulted in a too high expansion of the sludge bed, which reached the GLSS device.

The two lab-scale reactors presented very similar performances (data not shown). The main difference between the two reactors was that, logically, the one operated at the higher V_{up} showed a slightly higher loss of suspended solids to the effluent than the other (53 ± 94 against 37 ± 41 mg TSS/L). In both cases, the loss of TSS was however five to six times lower than that observed at full-scale whilst the COD removal was improved by 10 to 20%. The differences between the lab- and full-scale reactors were probably the result of the higher temperature at which the lab-scale experiment was performed (33.5 against 23 °C) but were considered not so important as to affect the analysis of the increase of the upflow velocity on the granulation process and its transposition to full scale.

The biomass of the reactor operated at 0.06 m/h did not show a positive evolution of its particle size distribution. Only a slight segregation of the particles within the sludge bed could be immediately noticed, the biggest granules being logically in higher amounts at the bottom of the reactor (data not shown).

The reactor operated at 0.5 m/h presented also a segregation by size of the granules within its sludge bed, but much more noticeable than previously (Fig. 5). A positive evolution of the sludge particle size distribution was observed initially. For instance, on day 54 of operation, less than 40% of the sludge TSS corresponded to particles smaller than 0.23 mm at the bottom of the reactor, whilst they represented almost 80% of the TSS in the inoculum. Nevertheless, for the next sampling performed on day 70, the sludge particle size distribution had returned to the values found on day 22 (Fig. 5). On the same way, the granulometry of the sludge at the top of the reactor, which had improved during the 42 first days of operation started to deteriorate in the subsequent samplings. This deterioration occurred after a change in the quality of the wastewater used to feed the reactors which presented a higher content of organic matter (3000–4000 mg COD/L against 800 mg/L) as well as a thin layer of floating material with an oily appearance.

After 81 days, both reactors were emptied and their sludge beds were homogenised for a last granulometry determination. Surprisingly, no differences were observed. In addition, their particle size distributions remained almost identical to the initial one as well as their SVI (28.9 and 27.4 mL/g TSS for the reactors operated at 0.5 and 0.06 m/h against 26.5 mL/g for the inoculum). The length of the experimentation (almost 12 weeks) could possibly be too short to see any positive effect of an increase of the upflow velocity. Ten weeks had been however sufficient for a similar study performed with crushed granular sludge and sucrose, an easily biodegradable compound, as substrate (Arcand et al. 1994). At least this experiment showed that an increase of the upflow velocity had no detrimental effect on the performances of the reactors and the granulometry of their sludge.

Concluding remarks and perspectives

The factors responsible of the disintegration/lack of granulation in the present case study could not be identified, although the role of milk fat not removed in the DAF pre-treatment could not be completely discarded. It was shown that the granulation problem was not related to (1) a deficiency or excess of some macronutrients or micronutrients, (2) a high proportion of COD due to proteins or volatile fatty acids, (3) a high concentration of ammonium, (4) a high development of sulphato-reduction and (5) a sub-optimum selection pressure due to a low water upflow velocity.

The initiative to artificially granulate the sludge through the addition of a cationic polymer failed and instead caused a heavy sludge flotation. This shows that despite the excellent results reported in the literature at lab scale since three decades, it is premature to advise the dosage of such polymers to promote sludge granulation in running (biogas producing) full-scale UASB-like reactors. Their use should be accepted only if successful reports on their application at full scale and detailed procedures for their dosage become available. A more promising way to induce granulation with polymers may correspond to a process similar to the one patented by Biothane

(Frankin and de Pijper 2007). In this process, the sludge is pregranulated outside the reactor through the addition of an organic cationic polymer followed by the addition of an inorganic anionic polymer plus some additives (activated carbon, calcium, etc). Published results suggest that the granules obtained in such a way have excellent settling properties and keep their integrity (no degranulation) in the long-term operation of UASB reactors (Jeong et al. 2005).

Despite of being limited to a specific case, the present work has a broader perspective since it gives an example of most of the points that must be investigated when a granulation problem occurs, an example that is true for any type of wastewaters.

In the case under study, at the end of the survey, the lack of granulation was not a problem anymore since the SRT of the remodelled reactors was higher than 100 days (Table 2). This means that contrarily to the beginning, they had acquired an excellent capacity to retain the flocculent biomass, a capacity associated to stable and high COD removal.

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