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Impact of solids retention time on the biological performance of an AnMBR treating lipid-rich synthetic dairy wastewater

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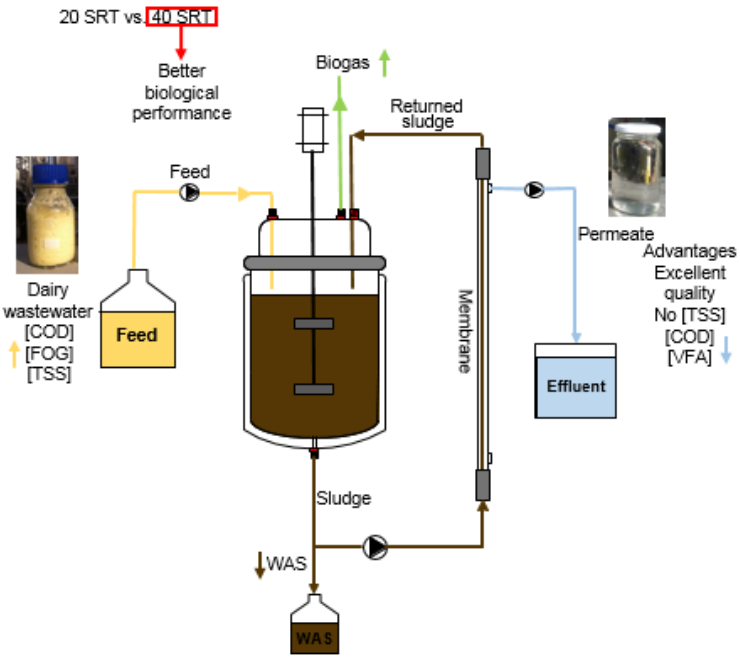
Abstract

In this study, the impact of applied solids retention time (SRT) on the biological performance of an anaerobic membrane bioreactor (AnMBR) treating synthetic dairy wastewater with high lipid content was assessed. Two side-stream AnMBR systems were operated at an SRT of 20 and 40 days (R20 and R40, respectively), equipped with an inside-out tubular membrane operated in cross-flow mode under full-scale operational conditions, i.e. crossflow velocity, transmembrane pressure, membrane flux. Successful operation was achieved and removal efficiencies of both reactors were up to 99% applying an organic loading rate (OLR) of 4.7 g COD L^{-1} d^{-1}. No precipitation of lipids was observed throughout the operational period, keeping the lipids available for the anaerobic degradation. Long chain fatty acid (LCFA) accumulation was very modest and amounted 148 and 115 mg LCFA-COD per gram of volatile suspended solids (VSS) for R20 and R40, respectively. At an SRT of 40 days, a slightly better biological conversion was obtained. Periodically performed specific methanogenic activity (SMA) tests showed stabilization of the SMA for R40 sludge, whereas for R20 sludge the SMA continued to decrease. This study revealed a more stable reactor performance operating the AnMBR at an SRT of 40 days compared to 20 days.

Graphical abstract



Cross-flow Anaerobic Membrane Bioreactor

Keywords: Anaerobic membrane bioreactor; Dairy wastewater; Lipids; Long chain fatty acids; Sludge retention time

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1. Introduction

The dairy sector produces large quantities of wastewater, approximately 0.2 to 10 liters of wastewater per liters of processed milk [1–3]. The main constituents of dairy industrial wastewater include easily biodegradable carbohydrates (mainly lactose), as well as proteins and lipids [4–6]. The exact composition of dairy wastewater considerably differs per location (Table 1), depending both on the type of dairy product being produced, such as milk, butter, yoghurt, ice-cream, desserts, and/or cheese, and on the production methods, operations, and technologies available at each particular industry. Most dairy wastewaters are characterized by considerable amounts of fats, oil and grease (FOG) (Table 1) [7]. Karadag et al. [8], reported FOG concentrations varying from 0.5 to 9.5 g L^{-1} and reported a detailed analysis of the long-chain fatty acids (LCFA) being present in dairy wastewater, mainly consisting of palmitic acid (23.5%), oleic acid (21%), and myristic acid (10.5%).

Table 1 – Dairy industrial wastewaters

Dairy industry	pH	COD ($g\ L^{-1}$)	BOD_5 ($mg\ L^{-1}$)	Solids ($g\ L^{-1}$)	Volatile solids ($g\ L^{-1}$)	Nitrogen ($mg\ L^{-1}$)	Phosphorus ($mg\ L^{-1}$)	FOG ($g\ L^{-1}$)	Reference
Cheese whey	4.9	68.6	7.71	1.95 (TS)	NA	1120 (TKN)	500	9.44	[37]
Ice-cream	5.2	5.2	2.45	3.9 (TS)	2.6	60 (TKN)	14	NA	[38]
Ice-cream	6.96	4.94	NA	1.1 (TSS)	0.99	NA	NA	NA	[25]
Milk processing	4.0-7.0	5-10	3-5	3-7 (TS)	NA	20-150 (TKN)	50-70	NA	[39]
Dairy	8-11	2-6	1.2-4	0.35-1 (TSS)	0.33-0.94	50-60	20-50	0.3-0.5	[40]
Mixed dairy processing	6-11	1.2-9.2	NA	0.3-1.7 (TSS)	0.3-0.8	14-272 (TKN)	8-68	NA	[7]
Cheese	5.5-9.5	1-7.5	0.6-5	0.5-2.5 (TSS)	NA	NA	NA	NA	[41]
Milk processing	NA	1.5-6	NA	0.3-2 (TSS)	NA	200-300 (TKN)	< 100	<0.5	(*)
Milk powder	NA	0.5-2	NA	<0.3 (TSS)	NA	<100 (TKN)	<100	<0.5	(*)
Fresh cream	NA	8-19	NA	7-8 (TSS)	NA	300-600 (TKN)	<100	0.1-0.3	(*)
Yoghurt	NA	5-20	NA	2-4 (TSS)	NA	200-400 (TKN)	0.2	0.3-1	(*)
Cheese	NA	2-13	NA	0.5-2 (TSS)	NA	200 (TKN)	0.1	0.3-1	(*)
Ice cream	NA	5-36	NA	5-10 (TSS)	NA	150-200 (TKN)	0.3	0.3-4	(*)

NA: Not available
FOG: Fats and Oil and Grease
COD: Chemical oxygen demand
(*) Internal data of Biothane-Veolia

The anaerobic treatment of industrial wastewater provides several advantages, such as high organic matter removal efficiencies, energy recovery through biogas production, and low sludge production and wastage [8, 9]. Dairy wastewaters have a high concentration of organics and lipids, being an ideal substrate for anaerobic treatment [10]. However, there are also negative aspects associated to the anaerobic conversion of lipids, which adds to the major complexity of treating lipid-rich wastewater such as dairy wastewater. During anaerobic

digestion, triacylglycerol lipids are firstly hydrolyzed to glycerol and LCFAs In general, hydrolysis of lipids occurs relatively fast, and the degradation of LCFA is considered the rate limiting step, potentially leading to the accumulation of LCFA in the system [11]. Even at low concentrations, the LCFA are toxic to methanogens and acetogens, whereby the unsaturated LCFA are more inhibitory than the saturated LCFA [12]. Moreover, LCFA adsorb onto the biomass causing mass transfer limitations affecting the biomass uptake of substrates and nutrients [13]. In addition, the adsorption of LCFA onto the biomass surface causes biomass flotation and washout, which particularly limits the application of sludge bed reactor systems such as the upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactor [14, 15].

Completely mixed reactor systems with a high biomass surface to liquid ratio are increasingly considered for the full-scale anaerobic treatment of FOG-rich wastewaters. However, the effectiveness of these systems fully depends on the effectiveness of the sludge separation device preventing sludge wash-out. Some systems combine an internal gas floatation unit for improved sludge retention such as the Biopaq® AFR reactor [16]. Other reactors rely on the complete retention of biomass using a membrane separation device [17]. At present, anaerobic membrane bioreactors (AnMBR) are indeed increasingly applied for the treatment of FOG-rich wastewaters such as dairy wastewater [18]. However, the required physical separation device is an additional and sometimes considerable cost factor to the anaerobic bioreactor. Therefore, process optimization is required that allows for minimizing the required filtration area in the membrane units. Previous research has shown that sludge filterability is determined by the prevailing sludge characteristics, which are impacted by the operational solids retention time (SRT) [19, 20]. In the treatment of lipid-rich wastewater, the SRT is considered a crucial operational parameter, because it will not only determine the degree of scavenged LCFA and thus extent of lipid conversion, but it will also determine the resulting specific methanogenic activities (SMA) of the sludge. The accumulation of LCFA in the system is directly related to the SRT or cell residence time of the biomass with contradictory effects: (i) slow growing microorganisms, such as those involved in the biodegradation of LCFAs would benefit from a high residence time in the system increasing the opportunities for degrading such compounds and reducing their accumulation in the system [10]; and (ii) the higher the SRTs, the higher the chances of accumulating LCFAs due to the reduced wastage of these compounds with the sludge waste. As a result, the SRT may significantly contribute to set the appropriate conditions for the accumulation or not of LCFAs in the system. Dereli et al., [19] reported a severe LCFA inhibition on the biological performance and methanogenic activity when working at 50 days SRT when treating corn-to-ethanol thin stillage; this is the only research reported in the literature relating the effects of the SRT to the anaerobic lipid degradation and LCFA accumulation in an AnMBR system. However, this research was performed with a very specific industrial wastewater with a different LCFA profile (corn-to-ethanol thin stillage), compared to dairy wastewater [10]. Moreover, the main conclusions of that study, such as the formation of round shape fat precipitates (called fat balls by the authors) and the biological inhibition when operating at high SRTs may be strictly related to that specific wastewater, making it very difficult to extrapolate such behavior to other types of wastewaters. Therefore, there is a need for a better understanding of the SRT effects on the biological performance of an AnMBR, fed with lipid-rich wastewater such as dairy wastewater. Our research directly addresses those needs.

The objective of this research was to evaluate the biological performance of an AnMBR treating synthetic (lipid-rich) dairy wastewater at different SRTs. In addition to assessing the overall performance of the anaerobic system, the impact of the presence and accumulation of LCFAs at different SRTs is evaluated.

2. Materials and methods

2.1. Synthetic wastewater

The synthetic dairy wastewater was prepared by diluting whole milk up to a COD and FOG concentration of approximately 10 g COD L^{-1} and 1.7 g FOG L^{-1}, respectively. Moreover, additional nutrients and micronutrients were added to the system [21]. The synthetic wastewater was prepared periodically (three times per week); the average wastewater composition for the entire evaluation is presented in Table 2.

Table 2 – Wastewater characterization

Parameter	Unit	Value
COD	g L^{-1}	10.1 ± 7.5
SCOD	g L^{-1}	3.3 ± 0.7
FOG	g L^{-1}	1.7
TS	g L^{-1}	6.0 ± 0.4
VS	g L^{-1}	5.4 ± 0.3
TSS	g L^{-1}	2.6 ± 0.5
VSS	g L^{-1}	2.7 ± 0.6
TKN	mg L^{-1}	273.5 ± 15.2
NH_3–N	mg L^{-1}	94.3 ± 0.3
TP	mg L^{-1}	27.6 ± 0.4

2.2. Reactor setup

Two AnMBRs were operated, each with an effective volume of 10 L equipped with a full-scale length (3 m) cross-flow tubular PVDF ultrafiltration membrane (Pentair X-Flow, The Netherlands) with a surface area of 0.049 m^2 and a mean pore size of 0.03 μm. The reactor was gently mixed at 35 rpm by a top entry mechanical mixer and via sludge recirculation with a recirculation pump. The reactor was fed by a peristaltic pump (Watson-Marlow, 120U/DV) from the influent tank. The filtration membrane was operated at a cross-flow velocity of 1 m s^{-1} applying a feed cycle of 890 seconds filtration and 10 seconds backwash. The backwash was done by reversing the flow of the peristaltic pump (Watson Marlow, 530S). Both reactors were double jacketed and a water bath was used to control the temperature at 35 °C. The pH was kept constant at pH (7.0 ± 0.5) using a pH controller. The biogas production was measured by a biogas flow meter (Drum-type gas meter Ritter, Germany). The entire reactor systems were controlled by a programmable logic controller (PLC) and the transmembrane pressure (TMP) was monitored throughout the operational time. The membrane filtration unit was operated at a flux of 10 L/(h^{-1} m^{-2}). The cross-flow velocity was set to 0.5 m s^{-1}. The operational TMP averaged at 300 mbar and 400 mbar for reactors R20 and R40, respectively. Figure 1 shows the reactor set-up.

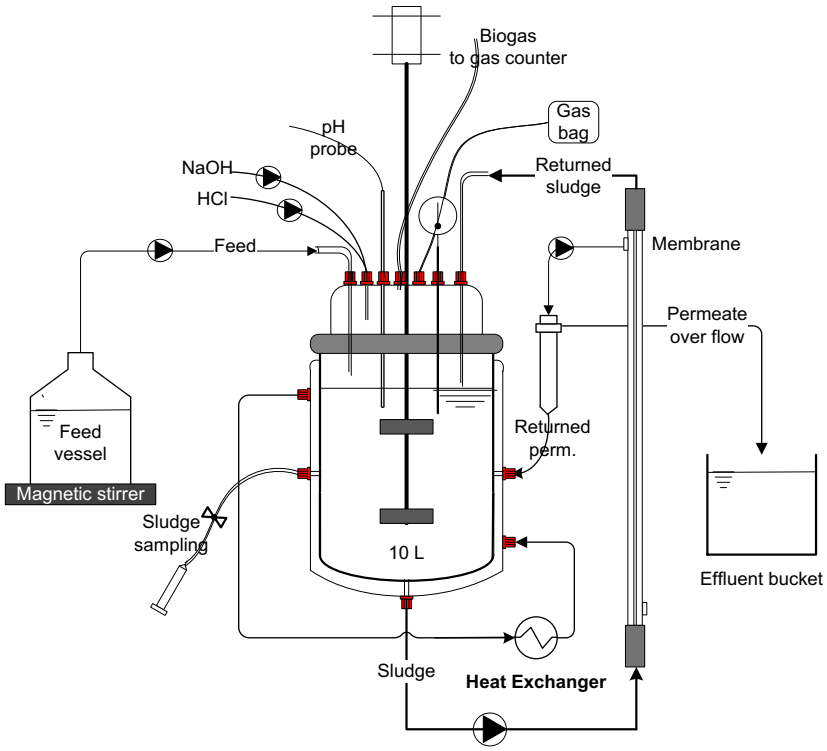

Figure 1 – Experimental set-up

2.3. **Experimental procedures**

The reactors were inoculated with crushed and sieved (600 µm mesh size) granular sludge from a full scale EGSB system (DSM; Delft, The Netherlands). Mesophilic conditions (35 ± 1) ºC were maintained. Both reactors were operated initially at an SRT of 30 days; the OLR was increased stepwise at 0.5 g COD $(L\ d)^{-1}$ every 5 days until reaching the targeted OLR of 4.7 g COD $(L\ d)^{-1}$. After 82 days of operation, the reactors were decoupled; hereafter, they were operated in parallel at different SRTs, i.e. 20 (R20) and 40 days (R40) for a period of 3 SRTs each. The reactors were operated at an HRT of 2.2 days. Once a week analyses were performed on the feed and the sludge, whereas and on the effluent, three times a week the following parameters were assessed: total solids (TS), suspended solids (SS), total Kjeldahl nitrogen (TKN) and ammonium nitrogen, which were measured according to Standard Methods of APHA of 1998. In addition, chemical oxygen demand (COD) and soluble COD were measured with Hach-Lange test kits. The volatile fatty acids (VFA) were analyzed by gas chromatography (GC, Varian 3900) equipped with a silica column (25 m and 0.53 mm internal diameter) and a flame ionization detector. Injector, column and detector temperatures were 250, 140 and 275 ° C respectively. Lipid content of the feed and sludge were determined by the norm ISO 1443. The individual LCFA composition of sludge were measured according to Neves et al. [22].

The specific methanogenic activity (SMA), using acetate, propionate and butyrate as the substrate was measured in sealed serum bottles (120 mL) by following the pressure increase with a pressure transducer (Centre Point Electronics PSI-30). The initial food mass ratio (F/M) of the tests was 1 g COD g^{-1} VSS. The liquid volume of the bottles was 50 mL and the biomass concentration was 2 g VSS L^{-1}. The anaerobic medium was prepared by dissolving sodium bicarbonate 3.5 g L^{-1} with tap water. The head space was flushed with a mixture of N_2:CO_2

(70:30%). The SMAs were carried out in batch tests using as substrates different volatile fatty acids (acetic, propionic and butyric acid). Linear regression of the slope of the methane production curve was performed and expressed as mg CH4-COD (g VSS d)$^{-1}$. The SMA experiments were performed every two weeks.

3. Results

3.1. Operational performance

Both reactors were kept at an SRT of 30 days for the first 82 days of operation, denominated as the "coupled period". In this phase the OLR was increased stepwise until reaching 3.5 g COD (L d)$^{-1}$. Afterwards both systems were decoupled and the OLR was increased up to (4.7 ± 0.7) g COD (L d)$^{-1}$ in R20 and (4.7 ± 0.8) g COD (L d)$^{-1}$ in R40. As can be seen in Figure 2a, throughout the entire evaluation (coupled and decoupled period), the COD removal efficiency of both reactors was higher than 99%, (99.3 ± 0.3) % for R20 and (99.6 ± 0.2) % for R40, and remained constant until the end of the experiment. That is, the biological performance of the systems was similar for both reactors. The effluent COD concentration was on average (67 ± 17) mg COD L^{-1} in R20 and (54 ± 10) mg COD L^{-1} in R40 (Figure 2).

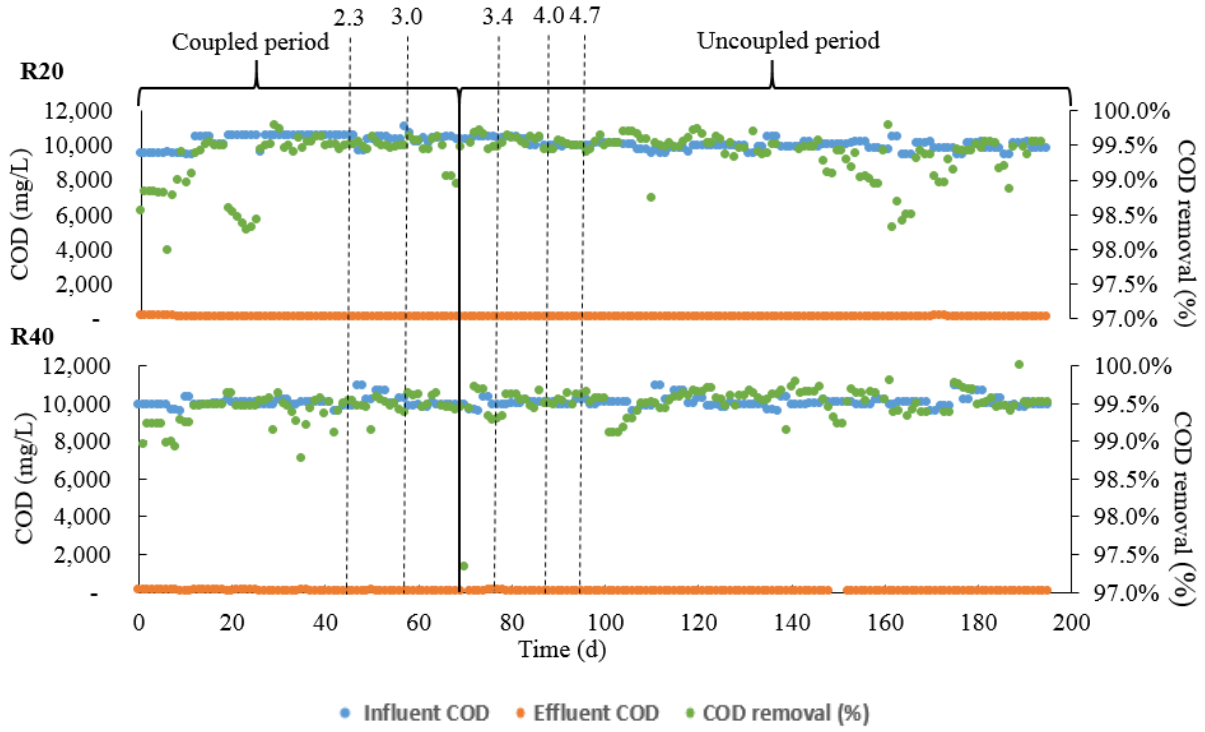

Figure 2 -Influent, effluent, and COD % removal throughout the operational time of R20 and R40 (dotted lines correspond to the OLR at the different stages).

The VFA concentration in the reactor/effluent is a good indicator of the anaerobic treatment performance; moreover, it can be used to monitor the activity of the acetogenic and methanogenic bacteria [15, 23]. Figure 3 shows the effluent VFA concentration as a function of the operation time of the reactors. At the beginning, in the coupled phase, the VFA concentrations average values of 3.3 and 0.3 mg VFA-COD L^{-1} for the R20 and R40, respectively. Then, after the decoupled period and up to an OLR of 4.7 g COD (L d) $^{-1}$, the VFA concentration increased in both systems at average values of 14 mg VFA-COD L^{-1} and 3.7 mg

VFA-COD L^{-1} for the R20 and R40 reactors, respectively. When both systems reached steady operational performance, at an OLR of 4.7 g COD (L d) $^{-1}$, the VFA concentrations in the effluent were 26 mg VFA-COD L^{-1} and 3.1 mg COD L^{-1} for the R20 and R40 reactors, respectively. GC analysis showed that the VFA composition was acetate, propionate and butyrate, with acetic and butyric acids being the major VFA constituents throughout the entire evaluation. As shown in Figure 3, an increase in the organic loading rate resulted only in a slight increase in the VFA concentration.

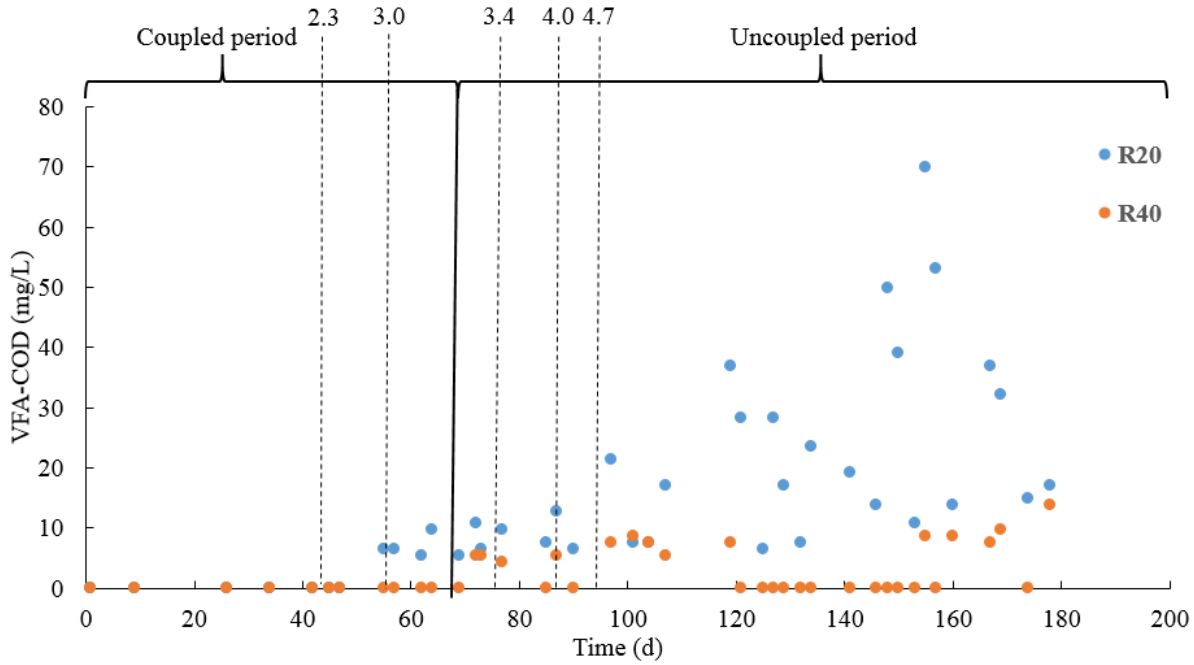

Figure 3 – VFA-COD effluent concentration over operational time

The specific methane production for the two reactors was on average 0.31 ± 0.02 NL CH_4 (g COD removed)$^{-1}$ and 0.32 ± 0.02 NL CH_4 (g COD removed)$^{-1}$ for R20 and R40 reactors, respectively. These values are lower than the maximum stoichiometric amount that could be obtained, i.e. 0.35 NL CH_4 (g COD removed)$^{-1}$. The small difference might be attributed to biomass growth (anabolic COD uptake) and some non-biodegraded COD that accumulates in the sludge.

3.2. **COD mass balance analysis**

The COD mass balance in both reactors showed negligible differences of 0.4% and 1.1% for the R20 and R40 reactors, respectively as shown in Figure 4 and Table 3. Dereli et al. [19] reported differences on the COD mass balance which were larger at shorter SRTs. They described the formation of aggregates in the sludge, described as LCFAs clumps (denominated 'fat balls' by the authors), that accumulated in the reactor at an SRT of 20 days and to lesser extent 30 days. At 50 days SRT these clumps were absent. Those particular sort of fat balls or LCFA clumps were not observed in our research.

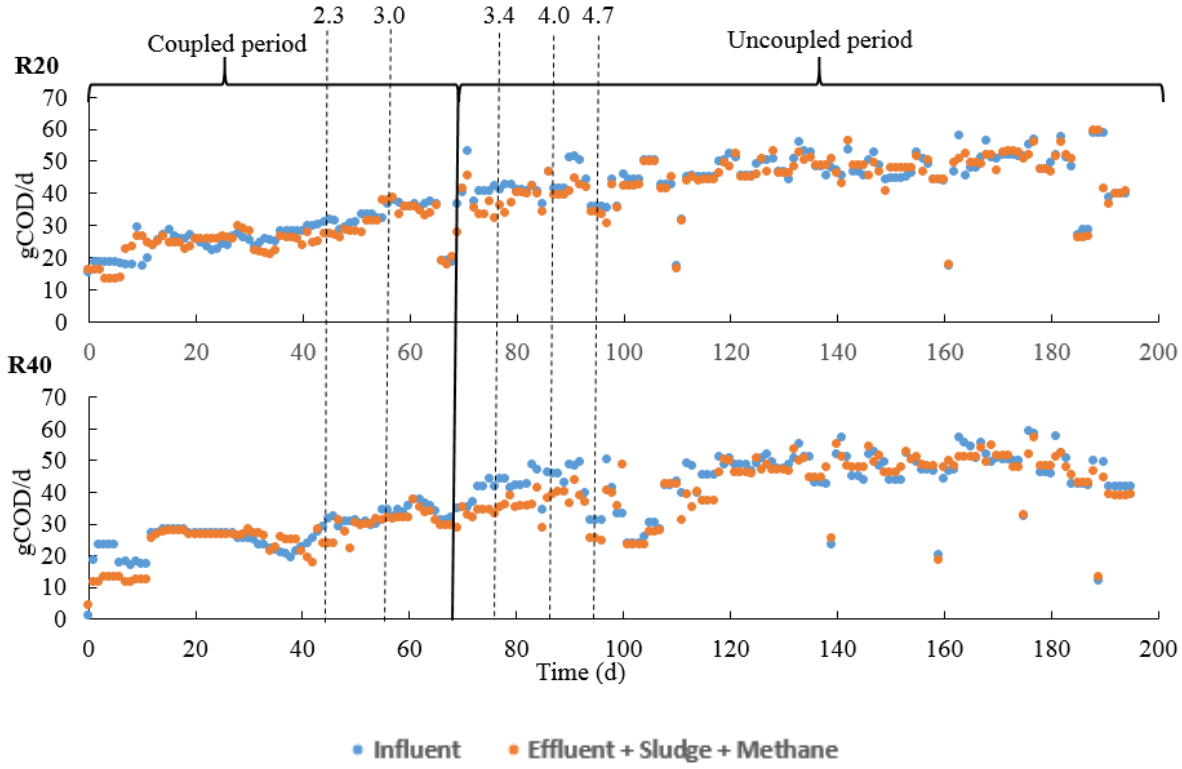

Figure 4 – COD mass balance

Table 3 – COD balance average at steady state

	R20		R40	
	g COD d^{-1}	%	g COD d^{-1}	%
Influent	47 ± 7		47 ± 9	
Effluent	0.3 ± 0.2	0.7	0.2 ± 0.1	0.5
Sludge	5.2 ± 0.4	11.0	4 ± 0.1	8.6
Methane	42 ± 7	87.9	42 ± 8	89.8
Total		**99.6**		**98.9**

The biogas production of both reactors produced under steady conditions was very similar, i.e. (15 ± 2) NL CH_4 d^{-1} and (16 ± 1) NL CH_4 d^{-1} for the R20 and R40 reactors, respectively.

3.3. **Total suspended solid concentration**

The total suspended solid concentration (TSS) was monitored throughout the operation of the reactors. As shown in Figure 5, the TSS concentration decreased at the beginning of the experiment for both reactors. Throughout the coupling period, when both reactors were kept at an SRT of 30 days, the TSS concentration was constant at (7.5 ± 0.5) g TSS L^{-1} and (7.6 ± 0.3) g TSS L^{-1} for R20 and R40, respectively. When both systems were decoupled and after reaching stable operation, the TSS concentration was constant at (6.8 ± 0.2) g TSS L^{-1} and (12.4 ± 0.4) g TSS L^{-1} for R20 and R40, respectively, until the end of the operational period. With respect to the VSS to TSS ratios, similar values were reported for both reactors of (0.93 ± 0.04) and (0.90 ± 0.02) for R20 and R40, respectively.

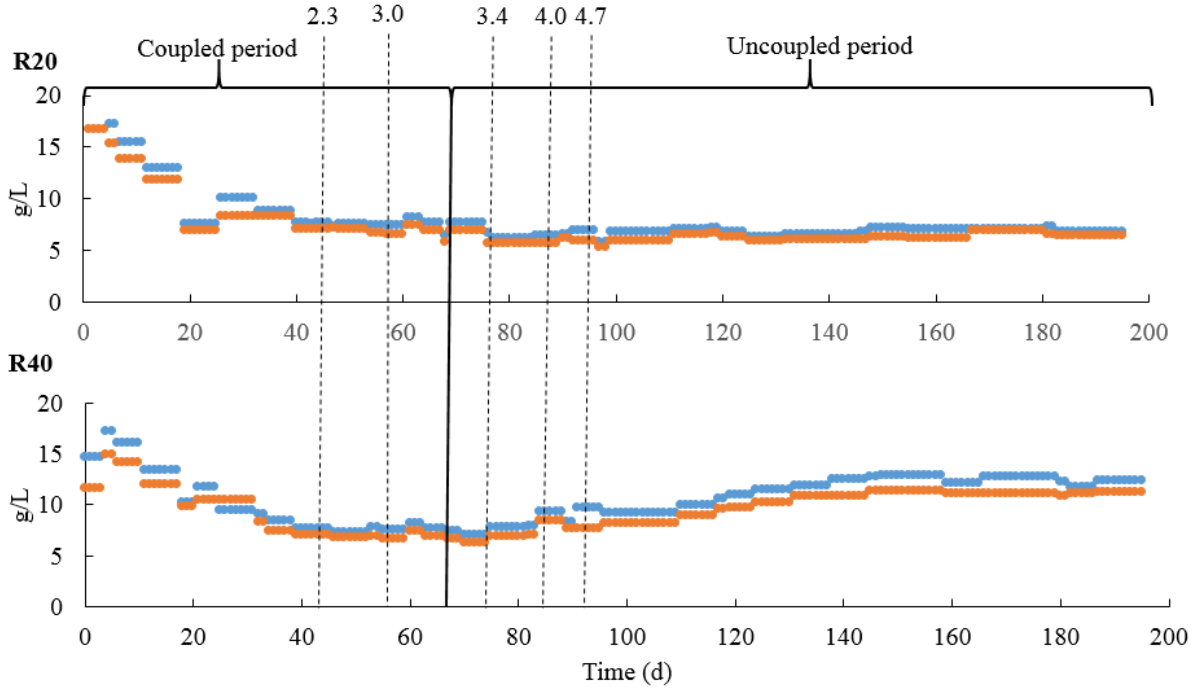

Figure 5 – Suspended solids concentration of both systems throughout operational time

3.4. **Sludge lipid concentration**

The lipid concentration of the sludge was determined on operational days 135 and 195 on both reactors to evaluate the potential lipid accumulation in the system; the results are presented in Table 4. The VSS specific lipid loading rates for the two reactors were calculated from the FOG concentration in the influent, the HRT, and the VSS concentration in each reactor for the R20 and R40 reactors; the values obtained were 0.13 ± 0.01 g lipid (g VSS d) $^{-1}$ and 0.073 ± 0.002 g lipid (g VSS d)$^{-1}$, respectively. The VSS specific lipid loading rates were relatively high, but similar to the values reported for instance by Dereli et al., [19], i.e. 0.10 – 0.04 g lipid (g VSS d)$^{-1}$. Considering that the influent lipid load (g lipid d^{-1}) to the reactors was the same for both reactors (R20 and R40) and that the VSS concentration was much higher for the R40 reactor, the R40 sludge experienced a lower VSS specific lipid loading rate.

Table 4 – Lipid content of the sludge per 100 g of mixed liquor (ML)

Lipid concentration	**g lipid (100 g ML)$^{-1}$**		**g lipid (g VSS)$^{-1}$**	
Operational day	**R20**	**R40**	**R20**	**R40**
132	<0.10	0.22	<0.16	0.20
195	0.10	0.15	0.16	0.13

3.5. **Long-chain fatty acid analysis in the sludge**

In both reactors the LCFA in the sludge were measured at the end of the operational period to determine a possible LCFA accumulation inside the reactors. Table 5 shows the detailed LCFA-COD composition determined in each reactor expressed per amount of mixed liquor (ML) and per gram of VSS in each reactor. In R40 the absolute concentrations of all LCFAs were higher than in R20 when reported as mg LCFA (g ML)$^{-1}$. However, when reported per gram of VSS, lower LCFAs concentration for R40 were compared to R20, as shown in the Table 5.

The most abundant LCFA types in both systems were oleic acid, i.e. 37% and 23% of the total LCFA for R40 and R20, respectively, and palmitic acid, i.e. 41% and 35% of the total LCFA for R40 and R20, respectively. The third most abundant LCFA was myristic acid, with a percentage of 19% and 13% in R40 and R20, respectively.

Table 5 – LCFA composition in the system expressed per amount of mixed liquor (ML) (results obtained from the 195 operational day)

LCFA concentration		**R20**		**R40**	
		mg LCFA-COD $(g\ ML)^{-1}$	mg LCFA $(g\ VSS)^{-1}$	mg LCFA-COD $(g\ ML)^{-1}$	mg LCFA $(g\ VSS)^{-1}$
Lauric acid (C12:0)		0.075	4.111	0.039	1.254
Myristic acid (C14:0)		0.370	20.398	0.547	17.115
Myristoleic acid (C14:1)		0.178	9.804	0.336	10.484
Pentadecanoic acid (C15:0)		0.023	1.265	0.043	1.344
Cis-10-Pentadecanoic acid (C15:1)		0.020	1.107	0.052	1.613
Palmitic acid (C16:0)		0.999	55.028	1.180	36.828
Palmitoleic acid (C16:1)		0.055	3.004	0.077	2.419
Stearic acid (C18:0)		0.212	11.701	0.194	6.093
Oleic acid (C18:1)		0.657	36.211	1.059	33.065
Vaccenic acid (C18:1)		0.066	3.637	0.090	2.867
Linoleic acid (C18:2)		0.026	1.423	0.047	1.523
Total LCFA	**mg LCFA-COD $(g\ ML)^{-1}$**	**2.68**		**3.66**	
	mg LCFA-COD $(g\ VSS)^{-1}$		**147.69**		**114.61**

3.6. **Activity of the sludge**

Throughout the entire operational period, the biomass activity was monitored for the two reactors by determining the SMA tests (Figure 6).

A decrease in the sludge activity was observed for both reactors, following the same trend. At the end of the operation of both reactors, the methanogenic activities on acetate, propionate, and butyrate decreased 26%, 77%, 50% for R20 and 46%, 13% and 14% for R40, showing a slightly higher decrease in the sludge activity on R20 compared to R40.

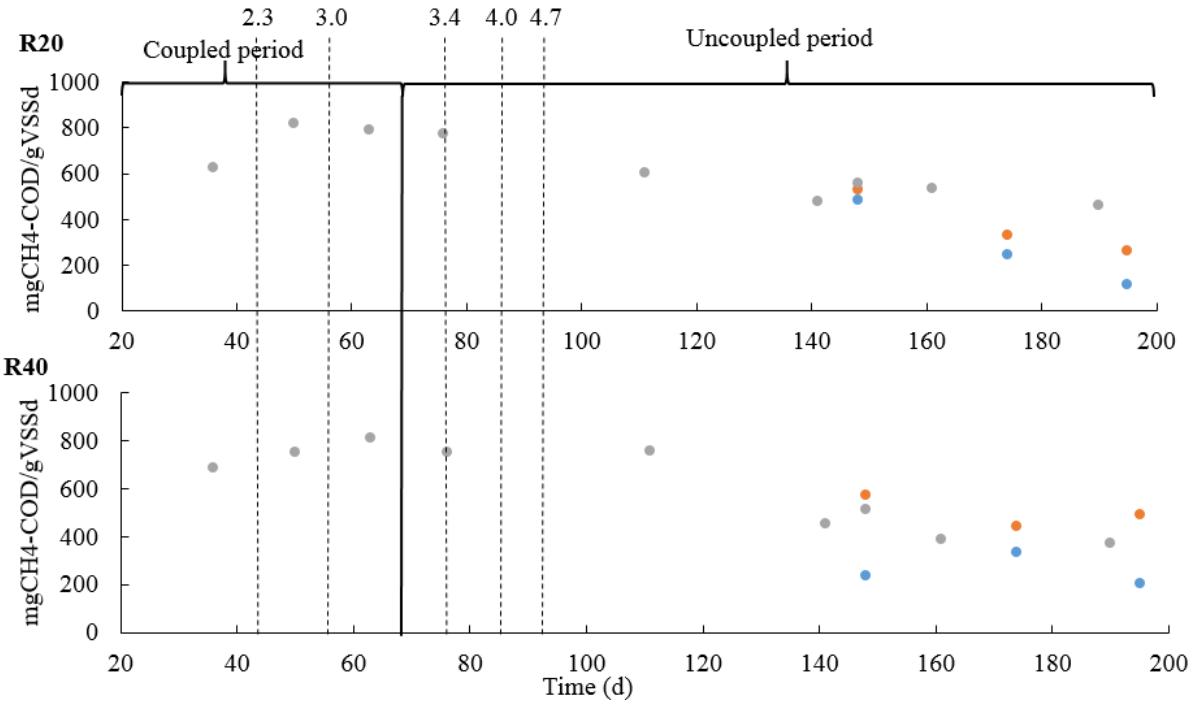

Figure 6 – Specific methanogenic activity for different VFA as function of the operational time of the reactors

4. Discussion

Both reactors operating at different sludge retention times were characterized by a stable operation indicated by both an organic matter removal of more than 99%, and by a stable biogas production; these performances were much better when compared to other studies on AnMBR treating other types of wastewater [19], and to other high-rate anaerobic wastewater treatment (HRAWT) systems [10]. Dairy industrial wastewater is complex to treat using sludge bed systems or and other HRAWT systems; the presence of fats in the wastewater induces sludge flotation and washout [24]. Hawkes et al. [25] studied the performance of a pilot scale UASB reactor treating ice-cream wastewater (lipid rich wastewater) at an OLR of 2 g COD $(L\ d)^{-1}$. The UASB system showed a poor performance with only 50% COD removal efficiency, mainly due to an unsuccessful granulation of the biomass in the system. Moreover, in the study of Rinzema et al. [26] complete sludge flotation was reported when treating lipid rich wastewater (a solution of capric and lauric acid) with a UASB reactor. Apparently, the AnMBR may present a good alternative to treat such complex wastewater, considering that the membrane physical barrier prevents the floating sludge to be washed out of the system. Moreover, several studies treating lipid rich wastewater using HRAWT [27, 28] reported lipid adsorption onto the sludge surface exhibiting mass transfer limitation; therefore, reducing the conversion rate to methane. In AnMBRs, the sludge is fully suspended and thus characterized by a very high surface area. Therefore, the lipids remain in the mixed liquor fully available to the microorganisms to be converted into methane. In addition, a higher effluent quality is obtained when working with an AnMBR, i.e. very low organic matter concentrations and free of suspended solids, compared to the effluent quality obtained with other HRAWT systems [29]. Such high effluent quality may introduce possibilities for water reclamation [30].

The COD mass balance fits very well for the both SRTs applied, i.e. 99.6% in R20 and 98.9% in R40, and the potential precipitation of lipids forming the so-called fat balls [19] was not identified throughout the operational time. The latter indicates that the lipids were entirely

available for anaerobic degradation. Effluent VFA concentration were slightly higher for R20 compared to R40 (Figure 3). When both systems reached steady operational conditions at an OLR of 4.7 g COD $(L\ d)^{-1}$, the VFA concentrations in the effluent were 26 mg VFA-COD L^{-1} (16 mg acetate L^{-1}, 3 mg propionate L^{-1}) and 3.1 mg COD L^{-1} (2 mg acetate L^{-1}) for the R20 and R40 reactors, respectively. That is, the reactors properly adapted to the OLR increase in a relatively short period of time. Nonetheless, a slightly better biological performance was observed for the R40 reactor, which might be attributed to the higher biomass concentration. Overall, the observed effluent VFA values in this study for both reactors were very similar and were much lower compared to the values reported for failing anaerobic reactors, i.e. 800 mg L^{-1} for acetic acid, propionic to acetic acid ratio 1.4, and butyric acid 5 mg L^{-1} [31].

Slightly higher digestion efficiencies were obtained at 40 days SRT compared to 20 days SRT. This is in accordance with reported values in the literature [32]. Higher biomass concentrations resulted in a slightly higher biodegradability. Moreover, a better effluent quality, a more stable performance, and more biogas production was obtained when working at high SRT. Also, the higher the SRT as in the case of the R40 reactor, the lower the sludge wastage. In fact, the degree of sludge stabilization increases with the applied SRT, leading to a reduction in the sludge treatment and management costs. The application of longer SRTs, such as in the study of Dereli et al. [19], who operated the AnMBR at an SRT of 50 days, resulted in a lower applicable OLR and therefore a higher HRT, compared to applied SRTs of 20 and 30 days. In that study the worst performance was observed at an SRT of 50 days [19]. The authors explained the better performance at the low SRTs by the formation of LCFA precipitates with cations forming fat balls, which has not been the case in our study. Very likely, by the formation of LCFA precipitates, less direct contact is experienced between LCFA and methanogenic biomass.

The applicable OLR and HRT in AnMBRs treating LCFA-rich wastewater depend on the achievable SRT and methanogenic activity of the sludge [42]. Literature data reveal that the applied HRT in AnMBRs treating lipid rich wastewater varies from 0.2 – 11 days [19, 43-45], all of them with COD removal efficiencies exceeding 95%. These results agree with our present results that show applicable HRTs of 2.2 days. Lipid hydrolysis proceeds relatively fast, whereas LCFA oxidation is known to be the rate limiting step in the anaerobic digestion of lipids [46]. This mismatch will result in the accumulation of LCFA in the reactor, possibly leading to perturbations. Morris et al. [47] treated slaughterhouse wastewater (lipid rich wastewater) in an anaerobic sequential batch reactor with HRTs ranging from 0.75 to 1.5 days with a SCOD removal of 90%. When lowering the HRT the TCOD removal decreased due to sludge flotation. The latter is a frequently observed problem in sludge bed reactors but is not apparent in AnMBRs due to the presence of an absolute membrane barrier. In our current research, results showed an excellent AnMBR performance applying OLRs and HRTs in a range similar to the discussed literature data. A further increase in OLR and/or drop in HRT is part of future studies. Taking into consideration the sludge lipid concentration, after 132 days of operation, R40 showed a higher VSS specific lipid concentration (0.20 g lipid $(g\ VSS)^{-1}$) than R20 (< 0.16 g lipid $(g\ VSS)^{-1}$). Possibly, the biomass in R40 was still not fully adapted for efficient lipids or LCFA conversion [33]. However, after 195 days of operation, R40 showed a lower VSS specific lipid concentration (0.13 g lipid $(g\ VSS)^{-1}$) than R20 (0.16 g lipid $(g\ VSS)^{-1}$).

Regarding the LCFA profile for both reactors, palmitic and myristic acid LCFAs showed the highest concentrations. Our observations agree with the research of Lalman and Bagley [12], who reported that palmitic acid (C16) and myristic acid (C14) are intermediates in the degradation of oleic and linoleic acids (C18). In addition, the oleic concentration was relatively high in both reactors, being higher in the R40 reactor than in the R20, which would be an indicator of an accumulation of oleic acid in the system. Oleic acid is an unsaturated LCFAs, which is considered more inhibitory for methanogens than the saturated LCFAs [12]. However, apart from a slight decrease in the SMA as explained below, our study showed no significant signs of inhibition regarding the biological operation in none of the reactors. The total LCFA that accumulated in both reactors was 2.7 and 3.7 mg LCFA-COD $(g\ ML)^{-1}$ for R20 and R40, respectively. These values were much lower than the ones reported in the literature, with values of 62, 48 and 61 mg LCFA-COD $(g\ ML)^{-1}$ for 20, 30 and 50 days SRT [19] at a similar influent lipid concentration of 1.7 g FOG L^{-1}. According to Pereira et al., [13], the inhibition of LCFA can be reversible between 1000 and 5000 mg LCFA-COD $(g\ VSS)^{-1}$; which are much higher values compared to the values obtained in our study, i.e. 147.69 and 114.61 mg LCFA-COD $(g\ VSS)^{-1}$ for R20 and R40, respectively. Very likely, the lack of mass transfer resistance in AnMBR systems results in an efficient LCFA conversion. Even though the reactor R40 was wasting less amount of lipids, the ratio LCFA-COD VSS^{-1} was the same or even lower compared to the reactor R20. Considering that the overall performance of both reactors was more or less similar, working at the highest SRT values is preferred as it add some additional advantages such as less LCFA accumulation.

According to Brockman and Seyfried [34], one factor to consider when operating a cross-flow AnMBR is the loss of sludge activity due to the disruption of the syntrophic association between the acidogenic and the methanogenic bacteria. It has been reported that high cross-flow velocities may exert a negative effect on microbial activity and cause a disruption of syntrophic associations operating a submerged AnMBR [35]. Moreover, the use of peristaltic cross-flow pumps in lab set-ups could result in a sludge milling effect, also contributing to the destruction of the syntrophic relationships between the different trophic microbial groups. However, in the study of Jeison et al. [36] no severe negative effect was found of the cross-flow induced shear rate on the acetogenic and methanogenic sludge activity. Results thus far, does not show evidence that the sludge methanogenic activity is negatively impacted by the applied cross-flow operation.

The results obtained from the sludge activity tests, showed a decrease in the SMA (Figure 4), even though the LCFA concentration measured in both reactors was lower than reported in previous studies [13, 19]. For the R20 sludge, the highest SMA was found when using acetate as the sole substrate; whereas for the R40 sludge, the highest SMA was obtained with butyrate as the sole substrate. Results indicate an effect of the applied SRT on SMA development and/or sludge composition. However, insufficient data hampers a clear interpretation of these findings. SMA development over time is in accordance to the study performed by Dereli et al. [19]. The inhibitory effect of LCFA on methanogenic and acetogenic microorganisms has been reported before [10]. According to Pereira et al., [12] the accumulation of LCFA in the system can lead to steric hindrance, or mass transport limitation, during substrate uptake leading to an SMA decrease. However, in our here-described studies, the decrease in the SMA was comparable for both reactors, so the applied SRT was apparently not discriminative. Regardless the results obtained concerning the SMA decrease, there was no sign of reactor perturbation, deterioration of biogas production, nor an increase in the effluent COD concentration. In addition, Vidal et

al. [1] reported SMA enhancement in the presence of lipids when butyrate was used as the co-substrate. This observation could also explain the higher SMA using butyrate as the substrate and the slightly better conversion of lipids in reactor R40. Figure 6 depicts a continuous decrease in SMA of the R20 sludge throughout the operational period when compared to the R40 sludge, which remained relatively stable. These results corroborate with the slightly higher lipid/VSS ratio found in the R20 sludge, due to the LCFA accumulation inside the system, which can negatively impact the sludge SMA [10]. For the R20 sludge, the SMA continued to drop, so it would be advisable to operate the system for a prolonged period of time in order to investigate whether a further drop will be experienced or an SMA stabilization at a lower level.

Considering the obtained results, it would be advisable to operate the AnMBR at an SRT of 40 days when treating lipid-rich dairy industrial wastewater: the sludge wastage can be minimized reducing the operational costs, and both the biogas production as well as the water quality of the treated effluent can be maximized.

5. Conclusions

• Lipid rich wastewater simulating milk processing industry wastewater with a lipid concentration of 1.7 g FOG L^{-1} was successfully treated in an AnMBR at different SRTs (20 and 40 days) with a stable performance regarding biogas production and COD removal efficiency during the operational time

• COD removal efficiencies over 99% and digestion efficiencies from 84% to 89% were obtained at an operational OLR of 4.7 g (COD L $d)^{-1}$ and an SRT of 20 and 40 days. The VFA concentration remained low in both systems (26 mg VFA-COD L^{-1} and 3.1 mg VFA-COD L^{-1} for the R20 and R40 reactors, respectively).

• After 195 days of operation, R40 showed lower lipid concentration (0.13 g lipid (g $VSS)^{-1}$) than R20 (0.16 g lipid (g $VSS)^{-1}$). The biomass seemed better adapted to lipids at high SRT.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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