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Exploring the limits of anaerobic biodegradability of urban wastewater by AnMBR technology

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Abstract

Anaerobic Membrane Bioreactors (AnMBRs) can achieve maximum energy recovery from urban wastewater (UWW) by converting influent COD into methane. The aim of this study was to assess the anaerobic biodegradability limits of urban wastewater with AnMBR technology by studying the possible degradation of the organic matter considered as non-biodegradable as observed in aerobic membrane bioreactors operated at very high sludge retention times. For this, the results obtained in an AnMBR pilot plant operated at very high SRT (140 days)

treating sulfate-rich urban wastewater were compared with those previously obtained with the system operating at lower SRT (29 to 70 days). At 140 days SRT the organic matter biodegraded by the AnMBR system accounted for 64.4% of the influent COD (45.9% was removed by sulfate reducing bacteria (SRB), and only 18.5% was converted into methane, highlighting the strong competition between SRB and methanogenic archaea (MA) when treating sulfate-rich wastewater). Almost half of the methane produced (46%) was dissolved in the permeate and most of it was recovered by a degassing membrane. The organic matter biodegraded by the AnMBR system was similar to the influent anaerobic biodegradability determined by wastewater characterization assays (68.5% of the influent COD), indicating that nearly all the influent's biodegradable organic matter had been removed. This percentage of degraded COD was similar to that obtained in previous studies working at 70 days SRT, showing that the limit of anaerobic biodegradability is was alreadyreached in this SRT. The organic matter considered as non-biodegradable according to wastewater characterization assays therefore was not seen to degrade in the AnMBR pilot plant, even at very high SRT. Once the biodegraded COD is close to the influent's anaerobic biodegradability, increasing the SRT is not justified as it only leads to higher operational costs for the same biogas production. These findings support the use of mathematical models for AnMBR design since they accurately represent the behaviour of these systems in a wide range of operating conditions.

1. Introduction

Water scarcity is becoming an increasingly severe global problem in need of urgent solutions. The new paradigm of focusing on recovering resources from municipalities has attracted increasing interest over the last few years.[1,2] Water and wastewater treatment plants represent 3-4% of Europe's energy consumption[3]. The implementation of anaerobic technologies in municipal wastewater treatment plants (WWTP) is a promising approach to re-using urban wastewater (UWW). UWW can produce biomethane, which helps to reduce the anthropological pressure on the environment and mitigates the WWTPs' carbon footprint.

Anaerobic treatments of UWW require practical and innovative technologies that can overcome the main drawbacks of these biological processes, such as the high Sludge Retention Times (SRT) required due to the low biomass growth rate and poor sludge settleability, which rule out biomass recycling systems. So far, anaerobic processes have been limited to urban wastewater in warm climates and highly loaded streams, such as industrial wastewaters or primary and waste WWTP sludge, in which the amount of methane produced per cubic meter of treated water allows operating temperatures to be increased. According to Martín *et al*. (2011), if the influent wastewater temperature is around 15 °C, the Chemical Oxygen Demand (COD) levels must be over 4–5 $g \cdot L^{-1}$ to generate enough biogas to raise the reactor temperature to 35 °C.[4]

Anaerobic Membrane Bioreactors (AnMBR) can overcome the drawbacks of applying anaerobic processes to low load wastewater. This technology combines an anaerobic reactor and a membrane filtration system, which achieves the complete retention of slow-growth microorganisms (no washout) and high SRT without increasing reactor volumes

(SRT and Hydraulic Retention Time (HRT) are decoupled) so that low load streams can be treated at ambient temperatures (mild climates) in AnMBR systems.

AnMBRs have several advantages over conventional activated sludge processes, including: (i) lower sludge production because of the low yield of anaerobic microorganisms. Jeison (2007) reported reductions of up to 90% in sludge production[5]; (ii) lower energy consumption because no aeration is required. According to Pretel *et al*., (2014) in mild/warm climates AnMBR technology could be a net energy producer when treating low sulfate-loaded wastewater[6]; (iii) lower greenhouse gas emissions, especially when methane is recovered from permeate[7]; and (iv) potential resource recovery because biogas energy and nutrient enriched fertigation water are obtained from the anaerobic degradation process.

Due to their advantages, interest in using AnMBRs for municipal wastewater treatment is on the rise.[8,9,2] One of the system's main issues is the dissolved methane present in the permeate, which can be up to 50% of the total methane produced.[10] The lower the temperature, the higher the amount of methane in the effluent. In this context, several methods have been applied, such as spray aeration towers, free fall jet towers[11], packed columns and tray aerators[12], diffused aerators[13], degassing non-porous membranes[14,15], or biological oxidation[16]. Degassing non-porous membranes have been found to be suitable for methane recovery from anaerobic effluents, achieving high methane recuperation levels and low energy requirements. Dissolved methane should be recovered from the effluent to reduce greenhouse gas emissions and improve the energy balance.[17]

Influent sulfate (SO_4^{2-}) concentration is another factor that significantly affects the energy balance in anaerobic processes. In anaerobic treatments, sulfate is biologically reduced to sulfide by sulfate-reducing bacteria (SRB). Proliferation of SRB in anaerobic reactors is considered undesirable because of the production of hydrogen sulfide (H_2S) instead of

methane. This compound also causes corrosion problems and can be inhibiting for both methanogens and SRB. Although all the SRB use sulfate as electron acceptor, these bacteria can be divided into two groups, according to the carbon source used. Autotrophic sulfate-reducing bacteria (ASRB) grow by using CO_2 as their sole carbon source and dissolved hydrogen as electron donor. These bacteria compete with hydrogenotrophic methanogenic *archaea* for dissolved hydrogen. On the other hand, heterotrophic sulfate-reducing bacteria (HSRB) use organic compounds as carbon source and electron donor. The preferred substrates for HSRB are low-molecular-weight compounds such as short-chain fatty acids and alcohols. HSRB compete with acetogenic bacteria and acetoclastic methanogenic *archaea* for volatile fatty acids (VFA) and acetate, respectively. Although SRB proliferation reduces methane production, these bacteria remove organic matter and thus contribute to meeting the effluent criteria.

SRT is one of the most important operational parameters in anaerobic membrane bioreactors: effluent characteristics, concentration of suspended solids in the reactor, biogas production, microbial community and degree of sludge stabilization, all depend on this factor. The higher the SRT the higher the biogas production and the level of sludge stabilization. However, high SRT also involves high suspended solids concentrations and membrane fouling for a given reactor volume.

The aim of this study was to assess the limits of the anaerobic biodegradability of urban wastewater by AnMBR technology. On this question, other authors have observed very low sludge production in aerobic MBRs operated at very high SRT.[18,19] This could only be explained by the degradation of suspended organic matter which should be considered non-biodegradable according to wastewater characterization assays. The aim was to determine whether this mismatch also occurs in AnMBR systems. Degrading the non-biodegradable organic matter would enhance methane production (improving the energy

balance) and would increase the economic feasibility of applying AnMBR technology to low loaded wastewaters. The results obtained in this work will contribute to improving the design of AnMBR systems, since they determine the amount of COD that can be degraded at different SRT. For this study, an AnMBR pilot plant was operated at very high SRT (140 days) treating sulfate-rich urban wastewater. The results obtained were compared with those obtained in previous studies at the same pilot plant at lower SRT (29 to 70 days).

2. Materials and Methods

2.1. Pilot plant description

Figure 1 shows a flow diagram of the AnMBR pilot plant where the study was carried out. It consists in an anaerobic reactor with a total volume of 1.3 m^3 (0.4 m^3 head-space volume) connected to two external membrane tanks (MT) of 0.8 m^3 total volume each (0.2 m^3 head-space volume), giving a total reactor volume of 2.1 m^3. Each MT includes an industrial hollow-fiber ultrafiltration membrane unit (PURON © Koch Membrane Systems (PUR-PSH31), 0.03 µm pore size, 31 m^2 filtration area). In order to control the temperature, the anaerobic reactor is jacketed and connected to a water heating/cooling system. A 0.5 mm screen size rotofilter (RT) is the pre-treatment system, followed by an 0.3 m^3 equalization tank (ET). To recover the dissolved methane from the treated wastewater a degassing membrane (DM) module is used, consisting of a non-porous polydimethylsiloxane (PDMS) commercial module with 2.1 m^2 of useful area (PermSelect®, MedArray Inc. USA). The obtained permeate is stored in an 0.2 m^3 Clean-In-Place (CIP) tank.

The plant, located at the Carraixet WWTP (Valencia, Spain), was fed with municipal wastewater from the WWTP pre-treatment, which involves screening, degritting and grease removal. The sludge is continuously recycled through the external membrane tanks (MT) where the effluent is obtained by vacuum filtration. A significantly high sludge recycling flowrate was established to obtain proper mixing conditions. In order to minimize the cake layer, a fraction of the produced biogas is recycled to the membrane tanks from the bottom of each fiber bundle. Further details of this AnMBR plant can be found in Giménez *et al*. (2011).[20]

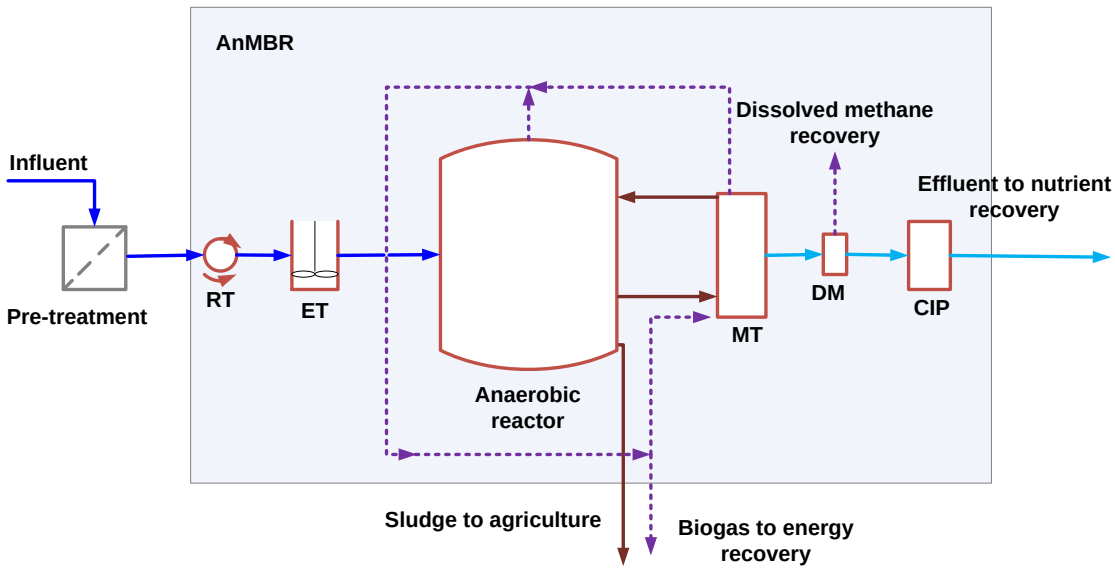

Figure 1 Process flow diagram (Nomenclature: RT: Rotofilter; ET: Equalization tank; MT: Membrane tank; DM: Degassing Membrane; CIP: Clean-in-place).

The plant is equipped with several on-line sensors and automatic equipment to monitor, control and automatize the plant operation. Redox, pH, temperature and pressure sensors are fitted to the anaerobic reactor to obtain information on-line on the performance of the process. Pressure sensors are installed in the filtration and degassing membrane tanks to

control the transmembrane pressure (TMP). Both the AnMBR plant control and the data logging are implemented on a SCADA system, thus centralizing all the different sensors and actuator signal in a single PC. Further information on this control system can be found in Robles *et al*. (2015).[21]

2.2. Experimental design

Table 1 shows the main operating conditions maintained in the pilot plant to study the AnMBR process performance at high SRT for approximately 9 months.

Table 1. Operating conditions of the AnMBR pilot plant

Operating Conditions					
Treatment Flow	**SRT**	**HRT**	**Temp**	**OLR**	**Qrec**
$L \cdot d^{-1}$	**d**	**h**	**°C**	**g $COD \cdot L^{-1} \cdot d^{-1}$**	**$L \cdot h^{-1}$**
2064 ± 23	140 ± 3	24.4 ± 0.4	27 ± 1	0.49 ± 0.10	1100 ± 20

The plant was operated at 140 days SRT and 24 hours HRT. The temperature was kept at an average value of 27 °C and the organic loading rate (OLR) was 0.5g $COD \cdot L^{-1}d^{-1}$. The recycled sludge flow rate (Q_{rec}) through the anaerobic reactor was set to 1.1 m^3 h^{-1} to obtain the proper mixing conditions.

Only one membrane module was required to obtain the established treatment flow rate. The different membrane operational stages consisted of: filtration (F), relaxation (R), backwash (B) degasification (D) and ventilation (V). During the experimental period, the membrane operating mode used under normal conditions was as follows: a 300 s basic

F–R cycle (250 s filtration and 50 s relaxation), 30 s of back-flush every 10 F–R cycles, 40 s of ventilation every 10 F–R cycles, and 30 s of degasification every 50 F–R cycles.

The biogas sparging intensity in the membrane tank was kept at 0.23 $Nm^3 \cdot m^{-2} \cdot h^{-1}$ (recycled biogas flow rate was established at 7 $Nm^3 \cdot h^{-1}$) to provide suitable shear conditions over the membrane surface.

The degassing membrane module was operated at a TMP of 0.8 bar to maximize methane recovery, pumping the AnMBR effluent through the shell side and recovering the permeate gas inside the fibers in order to reduce fouling drawbacks.

2.3. Analytical monitoring

In order to evaluate the performance of the biological process, samples of influent, effluent and anaerobic sludge were collected three times per week from the anaerobic reactor.

The following parameters were analyzed in the influent and effluent stream: total suspended solids (TSS), volatile suspended solids (VSS), total and soluble chemical oxygen demand (COD_T and COD_s, respectively), total and soluble biological oxygen demand (BOD_T and BOD_S, respectively), Volatile Fatty Acids (VFA), alkalinity (Alk), sulfate (SO_4-S), sulfide (S^{2-}-S), nutrients (NH_4-N and PO_4-P), Total Nitrogen (TN), filtered total nitrogen (TN_f), Total phosphorous (TP) and filtered total phosphorous (TP_f). The parameters analyzed in the anaerobic sludge were: total solids (MLTS), volatile solids (MLVS), COD_T, BOD_T, TN, and TP.

All the analyses were performed according to Standard Methods, except for carbonate alkalinity and VFA concentration, which were determined by titration according to the

method proposed by Moosbrugger *et al*., 1992.[22] BOD_T and BDO_S were determined using the experimental method based on the Warbug respirometer[23] using OxiTop experimental design (WTW), and Total Nitrogen was measured using standard kits (Merck, Darmstadt, Germany, ISO 11905-1).

AMPTS© (Automatic Methane Potential Test System, Bioprocess Control) was used to evaluate the influent wastewater anaerobic biodegradability and sludge digestibility. All the assays were carried out at a constant temperature of 35 °C without nutrient addition. To obtain both the biodegradability of the influent wastewater and sludge digestibility, the organic matter converted into methane in the experiments was estimated assuming that 350 mL of methane is produced from the degradation of 1 g of COD (theoretical value). The organic matter degraded by SRB in the tests was estimated assuming that 2 g of COD is degraded for each g of sulfate reduced to sulfide.

To determine the anaerobic biodegradability, duplicate tests were performed in hermetically sealed batch reactors of 2300 mL of capacity (300 mL head-space volume), and at Substrate / Inoculum volume ratio of 2 to avoid inhibitory effects (accumulation of volatile fatty acids). A single blank of each test was also prepared to determine the methane production of the inoculum (AnMBR sludge). This production was subtracted from the total methane production of the sample to determine net biogas production.

To determine sludge digestibility, tests were performed in duplicate for each sludge sample in a batch reactors of 500 mL capacity (100 ml head-space volume).

2.4. Dissolved methane determination

Effluent dissolved methane concentration was determined through the headspace method described by Giménez *et al*. (2012).[10] Liquid samples were collected once a week in 50

ml glass vials at the inlet and outlet of the DM module to determine the recovered dissolved methane. Tests were performed in duplicate. The vials were stored at 20 °C and continuously stirred for at least 4 h in order to reach gas-liquid thermodynamic equilibrium.

The methane content of the headspace vials was determined by gas chromatography, using a GC-FID flame ionization detector (Thermo Scientific). 0.1 mL of headspace gas was injected into a 30 m x 0.319 mm x 25 μmHP-MOLESIEVE column (Agilent Technologies) operated at 40 °C. Helium was used as the carrier gas at a flow-rate of 40 mL min^{-1} and pure methane gas (99.9995%) was used as the standard.

The performance of the degassing membrane module was assessed through the methane recovery efficiency (RE) defined as:

$$RE = \frac{[CH_4]_{dis.IM} - [CH_4]_{dis.OM}}{[CH_4]_{dis.IM}} \cdot 100 \quad eq.(1)$$

Where $[CH_4]_{dis.IM}$ and $[CH_4]_{dis.OM}$ are the dissolved methane concentrations determined at the membrane inlet and outlet, respectively.

2.5. Microbial characterization

2.5.1. Sample preparation

Sludge samples were collected from the reactor for the parallel viability and activity identification of relevant methanogens and sulfate-reducing microorganisms through a coupled viability and quantitative fluorescence in situ hybridization assay (viable qFISH).

Duplicate sludge samples were collected and diluted to a final concentration close to 4,000 mg $TS \cdot L^{-1}$ with fresh 1X phosphate-buffered saline (PBS). Cells were fixed with paraformaldehyde at 4℃ for 3 h and later rinsed with 1X PBS before long term storage in an ethanol: PBS mixture [1:1 (v/v)] at -20℃ until qFISH was performed.

2.5.2. Flow-cytometry viability assay

The viability assay was performed by flow-cytometry (FCM). Cells were disaggregated with 3 cycles of 40 s at Level 1 with Turrax and divided into 3 different 500 µL aliquots (blank, control and assay). SYBR green (10,000x) was used to detect cells in the sludge samples (control and assay) with a final concentration of 25X. After staining for one hour, Propidium Iodide (PI) was added at a final concentration of 0.02 $ng \cdot \mu L^{-1}$ to discriminate between viable and non-viable cells in the assay tube. Particles not targeted by either SYBR green or PI were related to biodegradable and non-biodegradable organic matter, as well as inorganic suspended solids present in the sludge. The FCM assay was performed by FACS Verse flow cytometer (BD Biosciences) and data were analyzed on BD FACSuite™ software (V1.0.6). The 30000 events analyzed were grouped according to their size and complexity by the forward scatter and side scatter detectors, respectively. The SYBR green signal was detected by excitation at a wavelength of 488 nm. The events detected with a positive signal (i.e. live and dead cells) were further analyzed by a 560 LP mirror and 586/42 filter that identified the PI-stained SYBR green signal of the events (dead cells). The remaining particles that were not stained with PI were assigned to viable events (live cells).

2.5.3. Quantitative fluorescence-in situ hybridization (qFISH)

Quantitative fluorescence-in situ hybridization (qFISH) was used to target viable microbial groups of interest. Details of the different probes used and the formamide (FA) percentages required to hybridize within samples are shown in Table 2. Probes were labeled with carboxyfluorescein 6-isomer (6-FAM) or Carboxytetramethylrhodamine 6-isomer (6-TAMRA) dyes, allowing signal detection of the different microbial group targets at 520 and 580 nm, respectively. A volume of 8 µL of paraformaldehyde-treated cells was fixed in gelatin-coated 10-well slides and later dehydrated with ethanol at 50, 80 and 98 % (v/v). Hybridization was performed at 46℃ for 1.5 h in a dark chamber with 1 µL from each probe and 8 µL hybridization buffer, prepared according to the required FA percentage (see Table 2). After hybridization the slides were placed in a warm washing solution at 48℃ for 15 minutes and air dried before microscopic quantification.

Table 2. Description of probes for microbial characterization used for quantitative fluorescence in situ hybridization.

Microbial group target	Probe	Oligonucleotide sequence (5'–3')	FA (%)	Dye	Reference
Archaea	ARC915	GTGCTCCCCCGCCAATTCCT	35	6-FAM: 6-TAMRA	[24]
Methanosarcinales	MSMX860	GGCTCGCTTCACGGCTTCCCT	45	6-TAMRA	[25]
Methanomicrobiales	MG1200b	CTGATAATTCGGGGCATGCTG	20	6-TAMRA	[26]
Methanobacteriales	MB311	ACCTTGTCTCAGGTTCCATCTCC	30	6-TAMRA	[26]
*Bacteria**	EUB338	GCTGCCTCCCGTAGGAGT	0-50	6-FAM	[27]
	EUB338-II	GCAGCCACCCGTAGGTGT	0-50	6-FAM	[28]
	EUB338-III	GCTGCCACCCGTAGGTGT	0-50	6-FAM	[28]
Desulfovibrionales	SRB385	CGGCGTCGCTGCGTCAGG	35	6-TAMRA	[27]
Desulfobacteraceae	SRB385db	CGGCGTTGCTGCGTCAGG	30	6-TAMRA	[29]

*The three bacteria probes were combined at equimolar concentration to target Bacteria domain.

**Dyes used were carboxyfluorescein 6-isomer (6-FAM) or Carboxytetramethylrhodamine 6-isomer (6-TAMRA)

An epifluorescence microscope (Leica DM 2500) equipped with a Leica DFC420c digital camera was used to quantify the percentage of microbial groups targeted on the fixed cells as follows. A minimum number of 20 images were taken and quantified with a custom script in MatLab software. The optimum threshold value required to detect each target according to their fluorochromes was used on all the images. The positive 6-TAMRA signal is the percentage of the pixel area detected in the area labeled with 6-FAM. The

final result is the mean positive percentage of the images analyzed. The statistical uncertainty was obtained by dividing the standard deviation by the square root of the number of microscopic fields analyzed.

3. Results and Discussion

3.1. Wastewater characterization

The pilot plant was fed with pre-treated urban wastewater. Table 3 shows the average influent composition (mean and standard deviation) for the whole operating period.

Table 3. Average influent composition

Parameter	Mean ± SD
TSS (mg·L^{-1})	342 ± 75
VSS (%)	79.5 ± 2.5
COD_T (mg COD· L^{-1})	510 ± 87
COD_S (mg COD·L^{-1})	104 ± 13
BOD_L (mg BOD·L^{-1})	359 ± 23
$BOD_{S,L}$ (mg BOD·L^{-1})	54 ± 7
BOD_5 (mg BOD·L^{-1})	305+-37
VFA (mg COD· L^{-1})	3.9 ± 2.5
Alk (mg CaCO3· L^{-1})	453.2 ± 34.6
SO_4-S (mg S· L^{-1})	119.2 ± 8.0
S^{-2}-S (mg S·L^{-1})	0
COD_T/SO_4-S (mg COD· mg^{-1} S)	4.2 ± 0.7
N_T (mg N· L^{-1})	52.8 ± 5.4
P_T (mg P· L^{-1})	10.2 ± 2.5
NH_4-N (mgN· L^{-1})	42.8 ± 3.4
PO_4-P (mg P· L^{-1})	5.5 ± 0.5
Aerobic biodegradability (%)	69.5 ± 3.3
Anaerobic biodegradability (%)	68.5 ± 2.8

In the data shown in Table 3, the high influent sulfate concentration (119.2 ± 8.0 mg $S \cdot L^{-1}$) should be noted in comparison with typical domestic wastewater (around 30 mg $S \cdot L^{-1}$). This concentration did not vary significantly throughout the study period, as shown by the low standard deviation. As already mentioned, a high sulfate concentration reduces the amount of methane produced since SRB usually outcompete methanogenic archaea (MA). In any case, since 2 g of COD are removed when 1 g of sulfate is consumed and the COD/sulfate ratio is above 4, a significant fraction of influent COD is available for methanogenic bacteria and produces a significant amount of methane.

It is also important to note that the influent aerobic biodegradability, (calculated as the BOD_L/COD ratio) is similar to the influent anaerobic biodegradability (calculated as the ratio between the organic matter anaerobically degraded in the anaerobic biodegradability assays and the total COD). This indicates that the organic matter's biodegradability does not depend on whether the treatment applied is aerobic or anaerobic. Important to highlight is that BOD assay is a more simple, accurate and standardized method than BMP assay. As aerobic and anaerobic biodegradability has shown similar values, BMP assays could be substituted by BOD assays. However, this fact should be verified with different wastewaters.

3.2. Pilot plant performance

The pilot plant was seeded with stored sludge (40% of the total work volume) from a previous operating period and operated for 255 days.

Figure 2 shows the time evolution of MLTS and MLVS concentrations in the AnMBR reactor during this period. MLTS concentration in the system increased from 7.5 $g \cdot L^{-1}$ on start-up to 20 $g \cdot L^{-1}$ when a pseudo-steady state was reached. As can be seen in Figure 2,

this concentration remained constant during the acclimatization period (first 40 days) in which the influent flow rate was progressively increased to avoid reactor acidification. Between days 40 and 165, both MLTS and MLVS significantly increased. Between days 167 and 205, operational problems due to biogas leaks and pumps out of service, coinciding with reduced influent organic load during a holiday period, reduced MLTS and MLVS concentrations. Around day 200, when influent organic load rose again and all the operational problems were finally solved, both concentrations started to rise again and reached a stable value around day 230. From day 230 to the end of the study period (vertical lines in Figure 2), MLTS, MLVS, effluent composition and biogas production did not vary significantly (less than 10%), indicating that a steady state had been reached, with MLTS concentration around 20 $g \cdot L^{-1}$ and MLVS around 13 $g \cdot L^{-1}$, giving an MLVS of 67%.

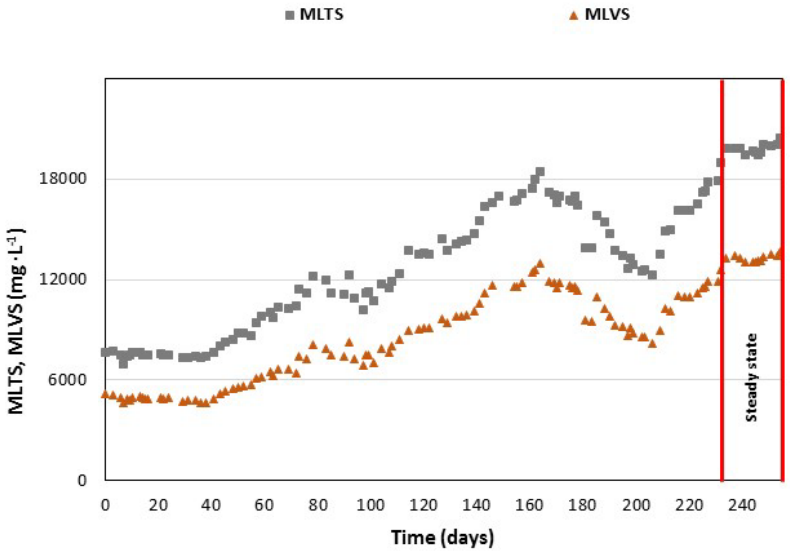

Figure 2. Time Evolution of MLTS and MLVS during the operating period.

3.3. Effluent characterization

Table 4 shows the average effluent concentrations obtained for different pollutants (mean and standard deviation) in the steady state period. As can be seen in Table 4, TSS-free effluent was achieved. The high efficiency of COD and BOD_5 removal (88% and 93%, respectively) met the discharge limits laid down by the European Wastewater Directive (CE 91/271). Effluent VFA concentration was negligible since the VFAs produced in the fermentation stage were consumed by MA or SRB. As shown in Table 4, all the influent sulfate was reduced to sulfide by SRB. Nutrient, ammonium and phosphate concentrations rose in the AnMBR effluent due to the mineralization of N- and P-containing compounds. The low organic content and high nutrient concentrations showed that the effluent obtained was suitable for reuse in fertigation. One of the main advantages of AnMBR technology is the possibility of recycling nutrients, decreasing the fertilizer consumption. This lower fertilizer consumption leads to significant energy savings: 19.3 kWh are saved per kg of nitrogen reused and 2.1 kWh are saved per kg of phosphorus reused[30]. Considering nitrogen and phosphorus concentration present in the effluent, energy savings account for 0.9 kWh/m3 reused for fertigation.

Table 4. Average effluent composition

Parameter	Mean ± SD
TSS ($mg \cdot L^{-1}$)	0
COD ($mg\ COD \cdot L^{-1}$)*	59 ± 10
$CH_{4dis.IM}$ ($mg\ COD \cdot L^{-1}$)**	44.9 ± 5.9
$CH_{4dis.OM}$ ($mg\ COD \cdot L^{-1}$)**	14.6 ± 2.4
BOD_5 ($mg\ BOD \cdot L^{-1}$)	14 ± 4
BOD_L ($mg\ BOD \cdot L^{-1}$)	26 ± 9
VFA ($mg\ HAc \cdot L^{-1}$)	2.0 ± 0.3
Alk ($mg\ CaCO_3 \cdot L^{-1}$)	817.3 ± 22.6
SO_4-S ($mg\ S \cdot L^{-1}$)	1.2 ± 0.6
S^{-2} (mg S·L-1)	113 ± 11
NH_4-N ($mgN \cdot L^{-1}$)	47.9 ± 7.6
PO_4-P ($mg\ P \cdot L^{-1}$)	6.7 ± 4.0

*COD does not include dissolved CH_4 concentration.

**$CH_{4dis.IM}$ and $CH_{4dis.OM}$ represent the dissolved methane concentration at the inlet and outlet of the degassing membrane, respectively.

Table 4 shows that the dissolved methane concentration in the permeate ($CH_{4dis.IM}$) was slightly below 45 mg COD L^{-1}. Taking into account the permeate flow rate, this concentration is equal to 35.3 L d^{-1}, which represents 46.5% of the total methane production. The degassing membrane was able to recover about 67% of the dissolved methane from the AnMBR effluent, being the dissolved methane concentration lost with the effluent ($CH_{4dis.OM}$) below 15 mg COD L^{-1}.Since the operational TMP was kept near to the highest value recommended by the manufacturer, methane recovery efficiency can be improved by adding more membrane modules. According to previous studies, dissolved methane removal efficiency can be increased by up to 80% with 2 modules and to over 90% by 4. Environmental and economical assessment is needed to optimize dissolved methane recovery process.

3.4. Biogas production

Table 5 shows average biogas flow rate and composition, jointly with the methane yield obtained (liters of CH_4 produced per g of COD removed). Considering the calorific power of methane (38000 KJ·m^{-3}) and the typical efficiency (35%) of a combined heat and power (CHP) device, the specific energy produced by the biogas flow rate obtained (71 L·d^{-1}) would be 0.073 kWh per cubic meter of treated water.

Unlike conventional anaerobic sludge digestion, significant variations in biogas composition and high N_2 concentrations were obtained, as can be seen in Table 5. Several factors were identified as influencing biogas composition, mostly related to influent physico-chemical characteristics such as COD concentration, COD/SO_4–S ratio and

wastewater temperature. The COD concentration and COD/SO_4–S ratio contribute to modify biogas composition by modifying CH_4, H_2S and CO_2 production. The influent temperature, especially the temperature gradient between influent and reactor, determine the amount of N_2 in the biogas, since, once in the reactor, the N_2dissolved in the influent is stripped out to the gaseous phase, contributing to dilute the biogas. In anaerobic treatments of low-strength wastewater, such as urban wastewater, high N_2 concentrations are achieved since less $CH_4$is produced per liter of treated wastewater[10].

It should be noted that the biogas flow rate shown in Table 5 does not include the dissolved methane recovered from the permeate. The biogas recovered by the degassing membrane (around 127 $L \cdot d^{-1}$) was not recycled to the head-space of the anaerobic reactor because of its high H_2S content (> 8%) and low methane content (< 25%). Case-by-case technical and cost studies would be required to determine the feasibility and the investment and operational costs of the different options for the biogas recovered.

Table 5. Biogas production, composition and methane yield.

Parameter	Mean ± SD
Q biogas ($L \cdot d^{-1}$)	71.0 ± 29.2
CH_4 (%)	57.2 ± 9.1
CO_2 (%)	6.8 ± 0.9
H_2S (%)	1.5 ± 0.3
N_2 (%)	34.5 ± 10.1
Y^{CH4} (L $CH_4 \cdot g^{-1}$ COD removed)	0.108 ± 0.018

The methane yield obtained was lower than the theoretical value (0.350 L $CH_4 \cdot g^{-1}$ DQO) due to the significant amount of methane lost with the effluent (46.5% of total methane

production) and to the high influent sulfate concentration, which reduces the organic matter available for methanogens.

3.5. Sludge characterization

Table 6 shows sludge production and characteristics and the results obtained from the sludge digestibility assays. The total solids concentration in the reactor reached a value of 20 $g \cdot L^{-1}$. Despite the high solids concentration, membrane fouling was not a problem because of the low transmembrane flux applied. The low percentage of MLVS (67%) is due to the high SRT value, which favors the proliferation of microorganisms responsible for particulate organic matter hydrolysis. The high SRT jointly with the low growth yield of anaerobic bacteria resulted in a low sludge production of 218 $gSV \cdot KgDQO^{-1}_{removed}$.

Table 6. Sludge characterization, sludge production and digestibility assays.

Parameter	Mean ± SD
COD_{sludge} (mg COD·L^{-1})	19405 ± 284
MLTS (mg·L^{-1})	19755 ± 364
MLVS (mg·L^{-1})	13228 ± 277
Sludge Production (gSV·$KgDQO^{-1}_{removed}$)	218 ± 6
Specific Sludge Production (g SV·d^{-1})	198 ± 4
MLVS (%)	67 ± 1
Aerobic Sludge Digestibility (%) *	33.8 ± 4.3
Anaerobic Sludge Digestibility (%)**	11.3± 0.9

*calculated as the sludge BOD_L/COD ratio; **measured in anaerobic assays carried out on Bioprocess

Sludge digestibility can be related to the degree of sludge stabilization. Very low values of anaerobic digestibility were obtained (11%) for the AnMBR sludge, which indicates that at this high SRT almost all the biodegradable organic matter was degraded by SRB or transformed into methane. On the other hand, the results of the aerobic digestibility are considerably higher (35%),which suggests the presence of organic compounds in the

AnMBR sludge that cannot be converted into methane but can be aerobically degraded. Further research is needed to explain the similarity of influent wastewater aerobic and anaerobic biodegradability (See Table 3) and the different aerobic and anaerobic sludge digestibility.

3.6. Microbial characterization of the viable reactor biomass

Microbial approaches based on targeting the ribosomes contained in the prokaryotic cells are an excellent way of detecting the presence of active groups in the biomass. Among these, FISH of molecular biomarkers such as the ribosomes offers a series of advantages over other techniques, such as the possibility of observing *in situ* cell morphology and quantifying its number or equivalent biovolume. The ease of application and the short time in which the results are obtained are other advantages of this technique. A coupled viability and qFISH assay was performed to determine the concentration of live active cells belonging to different microbial groups that played an important role during anaerobic digestion, such as methanogens or sulfate-reducers.

The scatter plots obtained from the flow-cytometry viability assays are shown in Figure 3. These assays determined that only 24% of the detected particles were viable or non-viable cells (Figure 3(a)). The remaining 76% could be assigned to biodegradable, non-biodegradable organic matter and/or non-volatile suspended solids. The viable cells were only 6% of the total particles analyzed, which gives a sludge viability percentage of 25% (Figure 3(b)). These percentages were so low because of the very high SRT. The negligible presence of viable cells was as expected, due to the previously commented high degree of sludge stabilization and low anaerobic sludge digestibility (see Table 6).

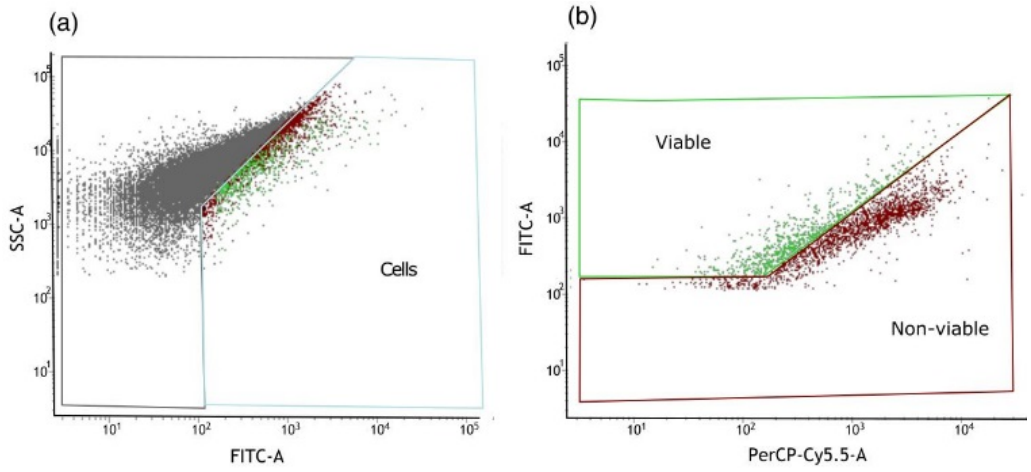

Figure 3. Flow-cytometry scatter plots representing the cells detected after SYBR green staining of the sludge (a) and viability discrimination after positive propidium iodide dyed cells (b).

Table 7 shows the results obtained from qFISH as compared with the results obtained by Giménez *et al.*,[20] in a previous study in the same pilot plant operated at a lower SRT (70 d). As can be seen, the present study found that 10% of the viable cells were MA, while SRB accounted for 4%. The ratio *Archaea:Bacteria* here determined was 10:90, coinciding with the values obtained by Regueiro and co-workers[36] when analyzing several mesophilic full-scale digesters with the same metathanogenic-specific probes. The predominance of methanogenic order *Methanosarcinales* (9%) has been previously observed by other authors[37,38] also using FISH technique in anaerobic digesters. The results obtained in the present study suggest that: (i) the methane in this AnMBR is mainly produced through acetoclastic methanogenesis; (ii) a high SRT favors this group, since the percentage is significantly higher than the one obtained at 70 d (Table 7), while the rest of the detected MA orders remained constant.

Regarding SRB, both detected groups (*Desulfovibrionales* and *Desulfobacteraceae*) were present in similar quantities (around 2%), similar to the results obtained in Giménez *et al.*, (2011)[20], suggesting that SRBs are not dependent on SRT for the range of SRTs

studied (70 – 140d). These two groups of SRB have been detected also in other studies (Reyes 2015) as the predominant SRB in anaerobic digesters.

Table 7. Microbial characterization FISH results obtained in this and previous studies

	Methanosarcinales	*Methanobacteriales*	*Methanomicrobiales*	*Desulfovibrionales*	*Desulfobacteraceae*
Specific probe	MSMX860	MB311	MG1200b	SRB385	SRB385db
This study	9±1	< 1	1±1	2±1	2±1
Giménez *et al.*, 2011[20]	5±1	1±1	1±1	less than 1%	less than 1%

Although the FISH technique has proven to be adequate to identify specific groups of microorganisms in sludge samples, the limitations of the technique (insufficient ribosomal content in cells, inaccessibility of the ribosome, autofluorescence background of the sample or probe availability) must be considered. For a wider and deeper study of the microbial populations involved in these processes, the use of other types of more current techniques is suggested (e.g. high-throughput sequencing of target genes such as the 16S rRNA). However, this type of study is beyond the scope of the present study.

3.7. Exploring anaerobic biodegradability through AnMBR technology

Table 8 shows the percentage of COD removed (COD_{rem}), biodegraded COD (COD_{deg}) and COD converted into methane (COD_{CH4}).

Table 8. COD Balance

Parameter	Mean ± SD
COD_{rem} (%)	88.0 ±4.3
COD_{deg} (%)	64.4 ± 4.1
COD_{CH4} (%)	18.5 ± 1.7

As can be observed, a high COD removal efficiency was achieved (88%). The difference between the removed (COD_{rem}) and biodegraded COD (COD_{deg}) is due to the COD withdrawn with the waste sludge. The table also shows that most of the COD degradation

was carried out by SRB, as the COD converted into methane (COD_{CH4}) only accounts for 18.5% of the influent COD. Comparing the biodegraded COD percentage (COD_{deg}) with influent wastewater anaerobic biodegradability (68.5%, see Table 3) it can be seen that almost all the biodegradable organic matter was degraded by SRB or converted into methane, which is in conformity with the low anaerobic sludge digestibility (11.3%).

Figure 4 shows the COD balance applied to the AnMBR system. The degraded COD is mainly due to SRB (45.9%), while the COD converted into methane only accounts for 18.5%, distributed between the methane in the biogas (9.6%) and the methane dissolved in the effluent (8.9%). It should be noted that the latter fraction is reduced by up to 2.9% when the methane recovered by the degassing membrane is considered. Also, a significant fraction of the influent COD (23.6%) was withdrawn with the waste sludge (COD_{WS}). The effluent COD (COD_{Eff}) accounts for 12% of the influent COD.

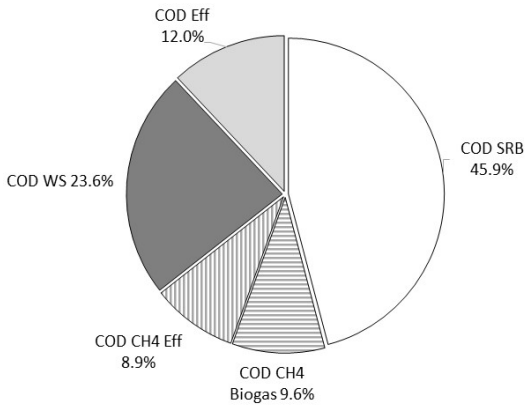

Figure *4*. AnMBR COD balance

Table 9 compares the anaerobic biodegradability results obtained in the present work with those obtained in previous studies in the same AnMBR plant at different temperatures and SRT. As can be seen, when SRT was increased from 29 days to 70 days, degraded

COD also rose from 34 to 62%. However, when SRT was extended from 70 to 140 days, the biodegraded COD percentage obtained in the present study did not vary significantly. It should be noted that the higher temperature of the 70d SRT study (33 ºC) also favored anaerobic degradation. These results suggest that the limit of anaerobic biodegradability had already been reached at 70d, indicating that operating the plant at very high SRT does not degrade the non-biodegradable part of the suspended organic matter, as has been observed in aerobic MBRs[18,19].

Since the temperature affects biological processes rate and the amount of COD degraded[32, 39] the SRT required for reaching anaerobic biodegradability limits depends on the reactor temperature. Further research will be needed to determine this SRT value at different temperatures.

Table 9. Anaerobic biodegradability in the AnMBR pilot plant at different operatingconditions

	SRT (days)	**T (ºC)**	**%COD_{deg}**
This study	140	27	64
Giménez *et al*., 2012[10]	70	33	62
Giménez *et al.,* 2014[32]	41	29	46
Giménez *et al*., 2014[32]	40	24	47
Giménez *et al*., 2014[32]	29	17	34

These results indicate that, despite the high SRT, the organic matter considered non-biodegradable by wastewater characterization assays did not in fact degrade. It can therefore be concluded that when biodegraded COD is close to the influent anaerobic biodegradability, extending the SRT will only increase operating costs but not biogas production.

Raising the SRT implies an increase in MLTS concentration and a decrease in sludge production (See Table 10). Regarding the MLTS concentration, the increase in this parameter is one of the main operational problems of the membranes. The higher the MLTS concentration, the higher the biogas flowrate required for operating the membranes in subcritical conditions, which is a key point for avoiding irreversible membrane fouling[33,34]. Therefore, the higher the SRT, the higher the membranes operational and maintenance costs. As can be seen in Table 10, sludge production significantly decreased between 40 and 70 days of SRT because of the considerable increase in the amount of organic matter degraded. However, the sludge production was only slightly reduced when the SRT was increased to 140 days because at SRT=70 days nearly all the biodegradable organic matter was already removed. These results support the aforementioned conclusion of not operating AnMBR systems at SRT over the value that allows reaching the maximum anaerobic biodegradability.

Table 10. MLTS, MLVS and sludge production in the AnMBR pilot plant at different operating conditions.

	SRT (days)	T (ºC)	MLTS ($mg \cdot L^{-1}$)	MLVS ($mg \cdot L^{-1}$)	Sludge Production $gVS \cdot KgCOD^{-1}_{removed}$
This study	140	27	19755	13228	218
Giménez et al., 2012[31]	70	33	13199	8501	240
Giménez et al., 2014[31]	41	29	9541	6275	370

A case-by-case study is thus needed to determine the optimum operating conditions for methane production at a reasonable cost.

Finally, the present study validates previous simulation results of the AnMBR design methodology[6,35] since the non-biodegradable suspended organic matter was found to remain constant whatever the SRT.

Conclusions

This paper describes a study on an AnMBR pilot plant fed with sulfate-rich urban wastewater. The main conclusions drawn from the work can be summarized as follows:

- As expected, SRB outcompeted MA, reducing all the influent sulfate to sulfide and also the organic matter available for methanogens. With an influent COD/SO_4-S ratio of 4.2, SRB degraded 45.9% of the influent COD and only 18.5% of the influent COD was converted into methane.
- Almost half of the methane produced (46%) was dissolved in the permeate. The degassing membrane recovered 67% of the dissolved methane. The recovery efficiency could be improved increasing the membrane area.
- The organic matter biodegraded in the AnMBR system accounted for 64.4% of the influent COD. The degraded COD value was similar to that obtained in a previous study at the same plant operated at 70 d SRT at a temperature of 33ºC, indicating that the limits of anaerobic biodegradability had been reached in the previous study.
- The degraded COD percentage was slightly lower than the measured influent wastewater anaerobic biodegradability (68.5%), indicating that, due to the high SRT, almost all the biodegradable organic matter was degraded in the AnMBR pilot plant. Unlike the results reported by other authors on aerobic MBR systems, the degradation of organic matter considered non-biodegradable by wastewater

characterization assays was not observed in the AnMBR pilot plant. This validates using mathematical models for AnMBR design, since non-biodegradable organic matter will not be degraded even at very high SRT.

Conflicts of interest

The authors have no conflicts to declare.

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