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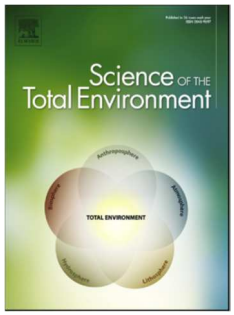

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Anaerobic membrane bioreactor for biogas production from concentratedsewage produced during sewer mining

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Abstract

A laboratory scale anaerobic membrane bioreactor was operated for 11 months treating synthetic wastewater that mimicked the concentrate from a forward osmosis process treating municipal wastewater with 80% water recovery. The effect of temperature variation on reactor performance was assessed. The reactor operated during 4 months at 34°C and then temperature was decreased to 23°C, 17°C and 15°C mimicking the typical temperature seasonal variations of the sewage. Average COD removal efficiencies were 95, 87, 76 and 67% at 34, 23, 17 and 15°C respectively, obtaining lower biogas production and lower COD removal at lower temperatures. Dissolved methane in the permeate averaged 8.2 mg CH_4/L and did not significantly change with temperature. After 2 months operating at 15°C, temperature was progressively increased, resulting in an immediate increase of methane production and COD removal efficiencies. Microbial analysis showed important changes in the archaeal community when temperature was changed from 34 to 23°C.

Keywords: Anaerobic membrane bioreactor; Biogas production; Municipal wastewater; Temperature

1. Introduction

The pressure on water resources as a result of city development has a negative effect on water systems, especially considering the extended use of high quality water for uses such as water irrigation and toilet flushing which do not require it (Bernal and Restrepo, 2012). In this context, the combination of centralized and decentralized treatment systems should be considered, not only to lower energy requirements but also to enhance water reuse. A combination of decentralized solutions with the already existing centralized systems is proposed as a valuable option in the context of water, energy and waste management. New sewer mining technologies for the treatment of wastewater and urban runoff water are currently being implemented mainly for water reuse in some countries where water scarcity is a major social and environmental concern (Wong and Brown, 2017). The main advantage of sewer mining is the production of reusable water where is needed, minimizing drinking water demands. In recent years, research has been directed into the development of emerging technologies, such as forward osmosis (FO), suitable for sewer mining to obtain reusable water. FO relies on an osmotic gradient driving force and a reverse osmosis membrane which allows high rejections of all contaminants which are concentrated in the reject stream while having low fouling propensity and requiring low energy for permeation, producing large volumes of clean water (She et al., 2016). The combination of FO with an anaerobic system could allow the recovery of not only clean water from the FO process but also the energy contained in the FO reject stream.

Anaerobic digestion has been traditionally implemented to treat high strength wastewaters or to digest sludge at mesophilic or thermophilic temperature ranges. However, during the last decade it has also been successfully implemented for municipal wastewater treatment at ambient temperatures achieving good removals in terms of organic matter, similar to aerobic treatment but with lower biomass production and smaller footprint (Bandara et al. 2011, Smith et al. 2013). This has been possible by using anaerobic membrane bioreactors (AnMBR) where the sludge retention time (SRT) is decoupled from the hydraulic retention time (HRT), avoiding

losing the slow growing anaerobic biomass.

The combination of FO and anaerobic treatment was introduced by Ansari et al., (2017) within the sewer mining concept. Low strength wastewater could be concentrated by FO up to approximately eightfold, significantly reducing the waste volume and increasing the chemical oxygen demand (COD). This increase in COD concentration makes the FO reject stream more suitable than municipal wastewater for anaerobic treatment, enhancing the recovery of energy in the form of biogas.

In recent years, several studies have examined the treatment of municipal wastewater with AnMBR, but only few have studied the effect of different temperatures on process performance (Gouveia et al. 2015; Miranda et al., 2015), being all of them conducted with municipal wastewater. Reported COD removal efficiencies vary from 95 to 76% when temperature is changed from 25 to 11 °C (Chu et al., 2005). Also, temperature has a direct effect on the solubility of methane, that increases when temperature is decreased (Crone et al. 2016; Giménez et al. 2011; Hu et al. 2006; Shin et al. 2014 Stuckey 2012). This not only limits the recovery of energy from the system but also poses a potential threat to the environment, with the release of this dissolved methane that can be fugitively emitted to the atmosphere, contributing to global warming.

This present study examines the effect of seasonal temperature variations on the anaerobic treatment of concentrated municipal wastewater with an AnMBR. It also assesses for the first time the challenges associated with the operation at relatively low temperatures (15ºC) and the recovery capability of this type of reactors once temperature increases. Changes within the microbial groups were also monitored.

2. Materials and methods

2.1. AnMBR setup and operation

A laboratory scale AnMBR of 5.4 L working volume was operated for 319 days (Figure S1). The reactor was connected to an external membrane module of 0.125 m^2 of membrane area

(0.15 L module volume. 2.1 cm internal diameter) built using polyvinylidene difluoride (PVDF) fibers of 0.4 µm nominal pore size of a ZeeWeed10 module (ZENON) in which the mixed liquor recirculation flow was kept at 0.83 L/min to avoid fouling problems without gas-sparging (4.6 cm/s of mixed liquor speed inside module). The reactor was equipped with pH and oxidation reduction potential (ORP) probes (CRISON) and a temperature sensor (SELECTA) and was continuously mixed at 35 RPM with a stirrer (RZR-1 Heidolph). Permeate flux was set at 2.1 LMH and was controlled by a peristaltic pump while reactor liquid level was controlled by a scale (KERN) which measured permeate volume. The reactor was inoculated with 2.5 L of anaerobic sludge (11 g VS/L) coming from the anaerobic digester of Girona's municipal WWTP which operated at 34 °C treating primary and secondary sludge. The sludge was diluted with 2.9 L of synthetic feed. A pressure sensor was positioned on the permeate line and when pressure reached values below -200 mbar, a back flushing of 20 seconds was applied. Membrane was manually cleaned by submerging it into a solution of 1000 mg/L HCL for 1.5 hours when frequencies of back flushing operations could not keep a constant HRT. Operational temperature was controlled with a water bath (Frigiterm. SELECTA) and was changed as described in table 1. Biogas volume was measured through a Milligas counter and collected in a Tedlar bag for the analysis of its composition. SRT was not controlled, being the biomass only removed from the reactor for analysis of solids and during the cleaning of the membrane.

2.2. Synthetic concentrated wastewater

Synthetic concentrated wastewater was made in order to mimic a concentrated medium strength municipal wastewater through a forward osmosis process with 80% water recovery (4 times original COD concentrating factor, 1.72 g COD/L)(Ansari et al., 2016). The concentrated synthetic wastewater composition used in this study was modified from Aiyuk and Verstraete 2004 (in mg/L): 312 of NH_2CONH_2, 39 NH_4Cl, 718 CH_3COONa, 86 Peptone, 60 K_2HPO_4, 26 $CaCl_2$ $2H_2O$, 134 $MgSO_4$ $7H_2O$, 60 Starch, 659 skim milk powder, 258 yeast extract, 144

soybean oil and 5mL of trace element solution. The trace element solution included (g/L): 3.8 $KCr(SO_4)_2$ $12H_2O$, 2.1 $CuCl_2$ $2H_2O$, 0.4 $MnSO_4$ H_2O, 0.9 $NiCl_2$ 6H2O, 0.4 $PbCl_2$, 1.0 $ZnCl_2$, 0.4 $FeCl_3$ $6H_2O$.

2.3. Chemical analysis

Total Solids (TS) and Volatile Solids (VS) were analyzed once per week according to the Standard Methods (APHA 1998). Phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) and ammonium (NH_4^+) were analyzed 2-3 times per week via ion chromatography (ICS5000, DIONEX). Volatile Fatty Acids (VFAs) and dissolved CH_4 were analyzed 2-3 times per week via gas chromatography (Trace GC Ultra ThermoFisher Scientific). Total COD concentrations in the feed and permeate were measured 3 times per week using test kits (Hach Lange, Dusseldorf, Germany). Total and partial alkalinity and IA/TA ratio were measured by pH titration (end points 5.75 and 4.3) using 0.1 N solution of H_2SO_4. CH_4 fraction in the biogas was daily measured with a Hydrocarbon detector Gir-3000 (GAS TECH, Australia) and once per month biogas composition was analyzed with gas chromatography (Trace GC Ultra Thermofisher Scientific).

2.4. Microbial analysis

Samples were collected on days 90 (34°C), 184 (23°C), 222 (17°C) and 272 (15°C) and stored at -20°C until DNA extraction. Total genomic DNA was extracted with FAST DNA Kit for Soils (MP Biomedicals, USA) following manufacturer's instructions. DNA concentration and purity were checked in all samples by Qubit fluorometer (Thermo Scientific) and Nanodrop 2000 UV- VIS spectrophotometer (Thermo Scientific) respectively. Genomic DNA from each sample was submitted to BMR Genomics (Padua, Italy) for sequencing. The V3-V4 hypervariable regions (Pro341F/Pro805R) of the 16S rRNA gene were amplified using universal prokaryotic primers (Takahashi et al., 2014), whose amplicons were sequenced on an Illumina MiSeq instrument (Illumina, San Diego, USA) using 2×300 bp paired-end reads. Sequences were quality trimmed using the MOTHUR software package (Schloss et al., 2009),

and aligned using the SILVA reference database (Quast et al., 2013). Subsequently, sequence libraries were randomly subsampled to contain the same number of sequences (14,374) for α- and β-diversity comparisons. The Ribosomal Database Project (RDP) pipeline and Classifier function were used to assign identities at a confidence threshold of 80% (Wang et al., 2007). Sequences were assigned to operational taxonomic units (OTUs) based on a 97% sequence similarity. Selected OTUs were also identified using the EzBioCloud database (Yoon et al., 2017). A heatmap showing the relative abundance of selected OTUs was generated using the gplots package in R, version 3.1.0 (http://www.r-project.org/). The Shannon diversity index (H') and the Chao1 richness estimator were also calculated as implemented in MOTHUR (Schloss et al., 2009). The unweighted and weighted UniFrac tests were applied to determine whether two or more communities have the same structure (Lozupone et al., 2007). Raw sequences have been submitted to the Sequence Read Archive (SRA) with Accession number PRJNA525616.

2.5. COD mass balance calculation

The COD mass balance was calculated for all the tested temperatures following equation 1.

$$COD_{tot} = COD_{CH_4 diss.} + COD_{CH_4 biog.} + COD_{biom.} + COD_{SO_4} + COD_{perm.} \quad \text{(Eq. 1)}$$

It was divided into the contribution of methane present in the biogas ($COD_{CH_4 biog.}$), the dissolved methane present in the permeate ($COD_{CH_4 diss.}$), the COD lost with the permeate ($COD_{perm.}$), the theoretical COD used for biomass synthesis ($COD_{biom.}$) and the theoretical COD used by sulphate reducing bacteria (SRBs) for sulfate reduction (COD_{SO_4}).

The COD due to the dissolved methane was calculated by converting the result of dissolved methane analysis of the permeate (1 $mgCH_4/L$ = 4 mgCOD/L (Foley, et al. 2015)). The contribution of methane present in the biogas to the COD mass balance was calculated following equation 2:

$$COD_{CH_4 biog.} = L\ CH_{4\ biogas}/Y_{CH_4} \quad \text{(Eq. 2)}$$

Where $LCH_{4biogas}$ are the liters of methane present in the produced biogas and Y_{CH4} is the theoretical COD/CH_4 yield and it is equal to 0.35 (Lier, et al. 2008).

The theoretical COD used for biomass synthesis was calculated following equation 3:

$$COD_{biom.} = Y \cdot gCOD_{removed} \cdot 1.42 \quad \text{(Eq. 3)}$$

Where Y is the anaerobic biomass yield and it is equal to 0.1 gVSS/$gCOD_{consumed}$ and 1.42 is the conversion unit of VS into COD (Lier et al. 2008).

The theoretical COD used by SRBs for sulfate reduction was calculated following equation 4:

$$COD\ for\ SO_4\ reduction = SO_{4\,removed} \cdot 0.67 \quad \text{(Eq. 4)}$$

Where 0.67 gCOD/gSO_4 is the COD utilization ratio by the SRBs (Lier et al., 2008).

3. Results and discussion

3.1. Reactor performance at different temperatures

The AnMBR reactor was operated for 319 days progressively decreasing the temperature from 34°C to 15°C and then increasing it back till 34°C to study the biomass recovery after 2 months of operation at low temperature (15°C). Reactor operation has been divided into five experimental periods depending on the operational temperature (Table 1). During the first period the reactor was operated at 34°C with an HRT of 30 hours resulting in an organic loading rate (OLR) of 1.30±0.12 This temperature was chosen to facilitate the adaptation of the anaerobic biomass (which was withdrawn from a local anaerobic digester working at 34ºC) to the operational conditions of the AnMBR. Under this temperature the reactor displayed high COD removal efficiencies (~95%) (Figure 1a). Also, reactor stability was confirmed by the Intermediate alkalinity/Total alkalinity (IA/TA) ratio which is a parameter commonly used to determine the stability of an anaerobic digestion process (Intermediate Alkalinity is given by the difference between Total and Partial alkalinity and it is an approximation of the VFAs concentration) (Basset et al. 2016; Bernard et al. 2018; Franco et al. 2015). An anaerobic

process is considered stable when the IA/TA is below 0.3 (Figure 1b). The VFAs concentration in the permeate was very small, averaging 16 mg/L as COD.

After temperature was decreased to 23°C on day 104, reactor continued stable (IA/TA<0.3) but its performance slightly deteriorated with average COD removal efficiencies decreasing to ~87%. VFA concentration in the permeate also increased resulting in an average effluent concentration of 124.1±70.8 mg/L as total VFAs (calculated using mg to COD conversion factor listed by Williams (1983)) during the 81 days of operation under this temperature. The first time in which the IA/TA parameter was higher than 0.3 occurred after temperature was reduced to 17°C on day 201. This was accompanied by an increase on COD and VFA concentrations in the permeate. This condition lasted for only the first 2 days after which stability (IA/TA <0.3) was reached again and COD removal efficiencies increased back to ~82% while maintaining the same OLR as in previous temperatures. When temperature was further decreased to 15 °C on day 222, COD removal efficiencies decreased until reaching an average COD concentration in the effluent of 684.1±165.0mg/L (average removal efficiencies of 58.4±10.1%). The IA/TA ratio increased exceeding 0.3 and reactor stability was compromised. In this case, the reactor did not recover and on day 256, the OLR had to be decreased to 0.92 by increasing the HRT from 29.6 to 43.9 h in order to avoid system failure. With this change, COD removal efficiencies recovered achieving 66.3±1.4% and VFA concentration in the effluent decreased to 357.5 mg COD/L as an average concentration. After 2 months operating at 15ºC, temperature was increased back to 23 and 34°C on days 280 and 296 respectively to assess the reactor recovery. COD removal efficiencies, IA/TA ratio and VFAs concentration in the permeate reached similar levels to the ones obtained in the initial periods at the same corresponding temperatures (Figure 1a & b).

Interestingly, temperature changes did not affect dissolved methane concentration in the permeate which stayed constant and reached supersaturation only in one case when operating at 23°C (Figure 1c). The average dissolved methane concentration during the whole operational

period was 8.2±4.9 mg CH_4/L. This differs from other studies that have shown higher dissolved methane concentrations when temperature is decreased (Smith et al. 2015). In our system, the low dissolved methane found in the permeate was probably due to the high mixing efficiency generated by the high flow of the mixed liquor recirculation in the membrane. Indeed our results are comparable to the values obtained by Yeo et al. (2013), in which, using a 5.7 L AnMBR with 1.6 L/min mixed liquor recirculation and additional gas sparging and working at 23°C, between 4.3±0.3 and 9.9±2.3 mgCH_4/L were found in the permeate in dissolved form. Methane fraction in the biogas was very high, always between 85 and 95%, independently of the temperature applied. Also high methane concentration in the biogas produced from an anaerobic reactor treating municipal wastewater and operating at 15°C was reported by Smith et al. (2015). They suggested that high methane content could be attributed to the low OLR applied, the high CO_2 solubility at the psychrophilic temperature and the feed composition in their system. In our case, however, we did not find any change in the methane content when the OLR was changed, being the methane percentage very high under all temperatures tested. This suggests that the high methane content might be attributed to the wastewater composition which was highly biodegradable.

Presence of VFA in anaerobic reactors is often associated to a warning signal in the process performance (Ahring, et al. 1995). The accumulation of VFAs occurs when acidogenesis is faster than methanogenesis and can cause inhibition to microbial groups responsible for methane production (Ngo et al. 2019). The presence of VFAs in the permeate during the operational period depended mainly on temperature changes. The average concentration of the most abundant VFAs produced (acetate and propionate) present in the permeate is shown in figure 2. A part of these two VFAs, isobutyric acid also started to accumulate at 23°C while n-butyric was present in the permeate exclusively when temperature was decreased to 15°C (Figure S2). However, their concentration was always below 15 mg/L.

3.2. Performance at 15 °C

Low temperature anaerobic digestion still represents an attractive option for reducing operational costs due to the high energy demand for keeping the anaerobic digester in the mesophilic or thermophilic temperature ranges (Lettinga, et al. 2001). In this study temperature was decreased to 15°C to study the effect that this temperature have on methanogenesis and system performance. When system temperature was dropped to 15°C, methane production rapidly decreased but never ceased. With the HRT set at 28 hours, system faced a gradual lowering of performance leading to system instability which was firstly evidenced by the IA/TA ratio that kept increasing reaching a value of 0.41 on day 256. There was a significant decrease of COD removal efficiencies from 80 to 43% because of the increase on the VFAs concentration in the permeate, especially acetic and propionic acids. Isobutyric acid concentration was always in the range (10-20 mg/L) and n-butyric acid concentration started fluctuating reaching values up to 40 mg/L. As reported in the study Ahring et al. (1995) the increase of n-butyrate and isobutyrate is associated with process instability. In our study, these VFAs were mainly present when operating the system at 15°C. In the case of isobutyric acid, its concentration was over 5.28 mg/L that was reported as a threshold for process imbalance (Hill and Holmberg, 1988). Despite the increase on VFAs in the effluent and the decrease on COD removal efficiencies, the reactor maintained its anaerobic activity and the production of biogas. This result contrasts with the study Dolejs et al. (2017) in which a decrease of temperature from 34 to 15°C in an AnMBR treating synthetic municipal wastewater led to a complete inhibition of the biological processes after two weeks of operation at this temperature, confirmed by the absence of both biogas production and VFA accumulation.

In order to restore process stability, the OLR was stepwise decreased from 1.44±0.02 to 0.92±0.03 by increasing the HRT from 28 to 43 hours from day 257. This increase of HRT was progressively applied during 3 weeks increasing it to 36 hours during the first week, 40 hours during the second week and to 44 hours in the third week. Methane production was constant even when the OLR was decreased, indicating that methanogenesis was the rate limiting step

in the process. The constant methane production at lower OLR improved the COD removal efficiencies from the reactor from 43% on day 256 to 60% (day 265), 63% (day 274) and 68% (day 282) which occurred simultaneously with a quick decrease in acetic and propionic acids concentration. Isobutyric and n-butyric acids were not affected by the change of HRT and their concentration was constant during the operation at 15°C. It is important to highlight that the stability of the reactor was compromised when temperature was decreased from 17ºC to 15ºC, suggesting this temperature as a minimum threshold under which the microbial processes are most severely affected.

3.3. COD mass balance

Figure 4 depicts the changes on the COD mass balance distribution under the different temperatures tested. The parameters which changed the most by changing the temperature were the contribution of methane present in the biogas and the COD lost with the permeate. Lower temperatures led to lower methane production and higher COD concentration in the permeate. COD mass balance during period II and IV (23°C) was very similar to results obtained by Sunaba et al. (2012) with an AnMBR treating synthetic wastewater at 25°C and 1.1 kg COD/m^3day of OLR. In their study the percentage of COD due to the dissolved methane was very low (3%) compared to the percentage of COD due to the methane in the biogas (72%) and was also attributed to the high mixing conditions through biogas recirculation which facilitated the release of dissolved methane to the gas phase.

The COD associated with biomass synthesis represented 14.1±0.3% of the total COD during the first period of operation and lowered with temperature to 12.9±0.5, 11.8±0.5 and 8.6±1.2 % at 23, 17, and 15°C respectively. When the temperature was increased the biomass synthesis also increased achieving similar results to the ones obtained at the beginning at the corresponding temperature (13.2±0.2% during period V and 14.2±0.1 during period VI). Sulfate reduction was constant and almost complete (95-99%), contributing to the mass balance in less than 1% of the total COD entering the system. Dissolved CH_4 contribution to the carbon

balance was also very low averaging 2% of the entering COD and remained constant despite the changes in temperature.

3.4. Microbial analysis

The microbial community was analyzed using high-throughput 16S rRNA gene sequencing to identify if changes in microbial community structure and composition were related to temperature changes. In general terms, the number of OTUs was the highest in period I (565), whereas other periods contained between 405 and 425 OTUs (Table S2). Shannon diversity index and Chao richness estimators also demonstrated that period I had a higher microbial diversity and richness than those samples collected at periods II, III and IV.

Differences in the microbial community composition were determined using the unweighted (sensitive to rarer taxa) and weighted (sensitive to abundances of taxa) UniFrac tests (Table S2), as implemented by MOTHUR, which showed that while samples taken during period III and IV had similar memberships (pairwise unweighted UniFrac test, p=0.933), the relative abundances of each OTU were different (Table S1). Bacteria was the dominant domain in all samples (Figure 5), with the most abundant phyla in sample of period I (34°C) being Bacteroidetes, Chloroflexi and Firmicutes. When temperature was lowered to 23°C, the greatest microbial community change took place, particularly in the relative abundance of the phylum Bacteroidetes which increased considerably at the expense of the phylum Chloroflexi and the archaeal community. Since operating at 23 ºC and for the lower temperatures phyla percentages did not change as much after the first temperature change and bacteroidetes, Firmicutes and Synergistetes were the most dominant. Candidatus Saccharibacteria relative abundance changed, increasing with decreasing temperature. *Desulfomicrobium escambiense* was detected at every temperature but at a very low abundance (always < 0.5%) and similar values were detected for every temperature (Figure S4). Probably this was due to the constant and not high concentration of sulfate. The percentage of the archaeal community compared to the overall microbial community decreased from 17% to 10% when lowering the temperature

from 34 to 23ºC remaining stable when further decreasing the temperature (Figure 5). Figure 6 shows the relative abundance of different archaeal species found within the archaeal community under each temperature. Four archaeal species were found, being two of them acetoclastic methanogens (*Methanothrix soehngenii* and *Methanosaeta* sp. represented by OTU 3 and OTU 8, respectively) and the other two hydrogenotrophic methanogens (*Methanospirillum hungatei* and *Methanobacterium subterraneum* represented by OTU 20 and OTU 22, respectively) (Figure 6). A shift in the predominant archaeal species was observed when temperature was decreased from 34ºC to 23ºC. At 34°C, *Methanosaeta* sp. (OTU 8) was the dominant species of the archaeal community with an abundance of 96.8 %. This percentage dropped to 7.9%, 1.1 % and 0.7% at 23, 17 and 15°C, respectively. This species was replaced by *Methanothrix soehngenii* (OTU 3), a mesophilic acetoclastic methanogen which belongs to the genus *Methanothrix*. Its abundance within the archaeal community increased from 2.9% at 34°C to 76.5, 86.5 and 85.4% at 23, 17 and 15°C, respectively. The other two archaeal species present in the AnMBR reactor were mesophilic hydrogenotrophic methanogens which slightly increased their percentage with lower temperatures. *Methanospirillum hungatei* (OTU 20) increased from 0.2% at 34°C to 9.4, 6.1 and 7.0% at 23, 17 and 15°C, respectively, while *Methanobacterium subterranum* (OTU 22) increased from not detectable levels at 34°C to around 6% of the archaeal community at other temperatures. Acetoclastic methanogens dominated in the AnMBR under all tested temperatures but the percentage of the hydrogenotrophic methanogens increased by 15% when lowering temperature from 34 to 23°C. This increase of the hydrogenotrophic population is in line with the study from Lettinga et al. (2001) that suggested that lower temperatures may not offer a considerable energetic advantage to the hydrogenotrophic methanogens. Overall, the decrease on methane production obtained at the lowest temperature tested and the increase on acetic acid concentrations confirms that acetoclastic methanogenesis is treated as a rate limiting step for methane fermentation process (Nozhevnikova et al., 2007).

Mesophilic psychotolerant populations dominated in the AnMBR making possible the increase of their activity once temperature was increased to 23 and 34°C as shown also by the rapid increase of treatment performance once temperature increased.

4. Conclusions

The treatment of concentrated synthetic municipal wastewater with an AnMBR at 1.38±0.25 gCOD/dayL^{-1} OLR at different operating temperatures was feasible and resulted in high levels of methane production. Reactor stability was compromised at 15ºC but was restored by reducing the OLR. Dissolved methane was under saturation level averaging 6.18 mgCH_4/L and remained constant across the different tested temperatures probably due to the high mixing efficiency applied. Microbial analysis showed mesophilic populations dominated in the AnMBR making possible the increase of their activity once temperature was brought back to 23 and 34°C, confirmed by the complete recovery of system performance.

5. Supplementary information

E-supplementary data of this work can be found in online version of the paper

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TABLES

Table 1. Process parameters and COD removal efficiencies at the different operating temperatures.

Operational period	Temperature	Removal efficiency (%)	Biomass concentration (g VS/L)	HRT (hours)	SRT (days)	Days of operation
I (0-119 days)	34°C	94.9±1.8	3.7±0.2	30.0±3,1	103	119
II (120-203 days)	23°C	86.6±4.6	5.1±1.3	28.4±2,2	83	83
III (204-224 days)	17°C	76.5±5.7	6.8±0.5	29.6±2,6	120	20
IV (225-284 days)	15°C	58.4±9.0	6,4±0.4	34.1±6.3	118	59
V (285-300 days)	23°C	87.9±3.3	5.7±0.0	34.8±6.0	100	15
VI (301-320 days)	34°C	95.1±0.9	5.4±0.2	24.7±4.8	100	19

FIGURE CAPTIONS

Fig. 1. Temporal variations of different monitored parameters during the operational period: **a)** COD removal efficiencies and OLR; **b)** Total VFAs concentration in the permeate and IA/TA ratio; **c)** dissolved CH_4 in the permeate. Lines indicate saturation level at the different operating temperatures, for 90 % CH_4 content in the biogas at 1.01 atm).

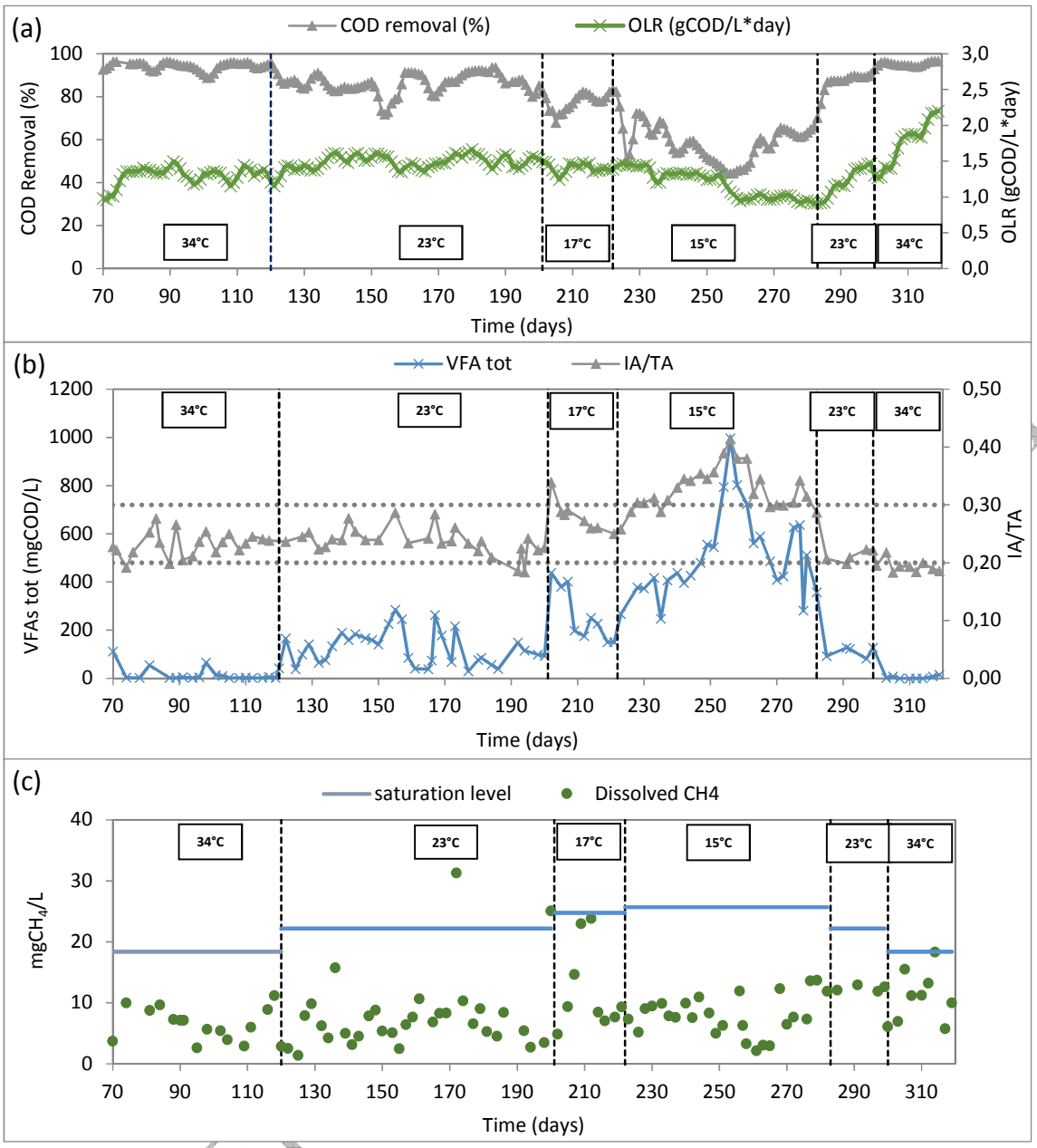
Fig. 2. Acetic and propionic acid average concentration with standard deviation at the different operating temperatures. (Daily values are reported in table S1)

Fig. 3. Temporal variations of different monitored parameters when operating at 15°C: a) COD mass balance; b) Acetic, propionic, isobutyric and n-butyric acid concentrations. (Temporal variations of COD mass balance and VFAs for the entire experiment duration are reported in Figure S3 a and b)

Fig. 4. Average COD mass balance under different operating temperatures.

Fig. 5. Relative abundance of archaeas and bacterias at phylum level.

Fig. 6. Percentages of archaeal species at the different the temperatures tested in this study.



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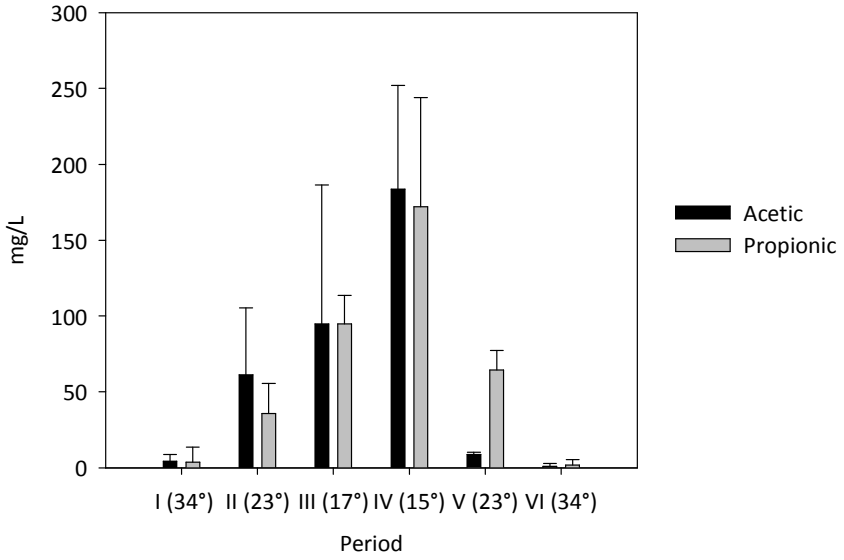


Fig. 2. Acetic and propionic acid average concentration with standard deviation at the different operating temperatures. Daily VFA's concentrations are shown in Table S1.

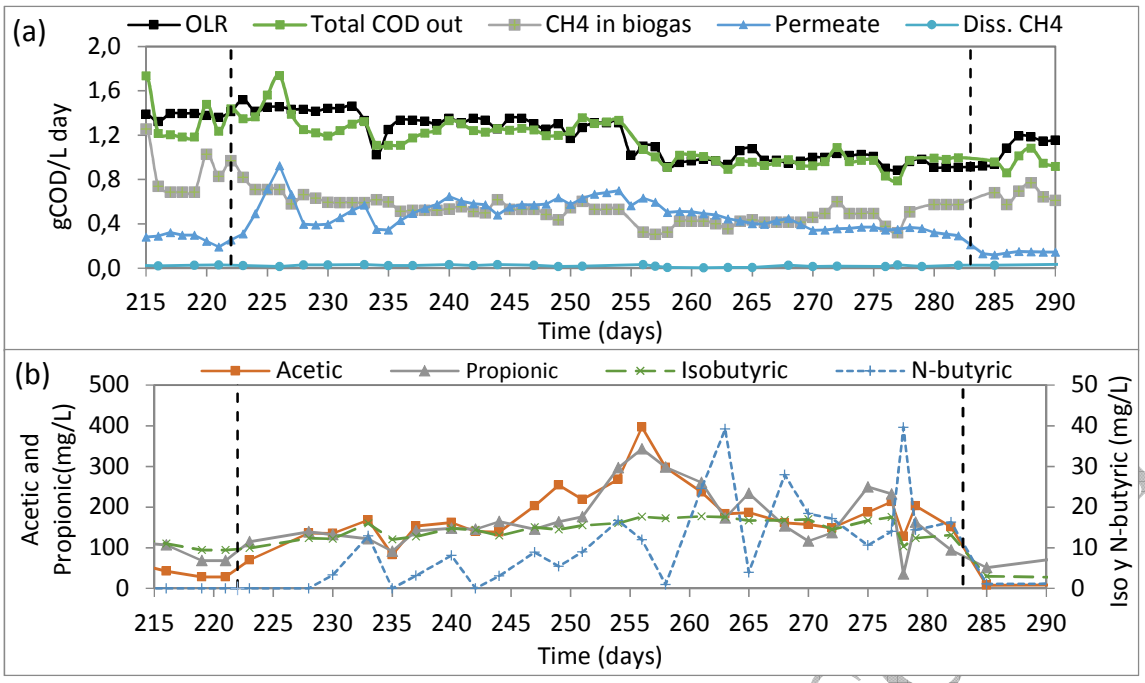
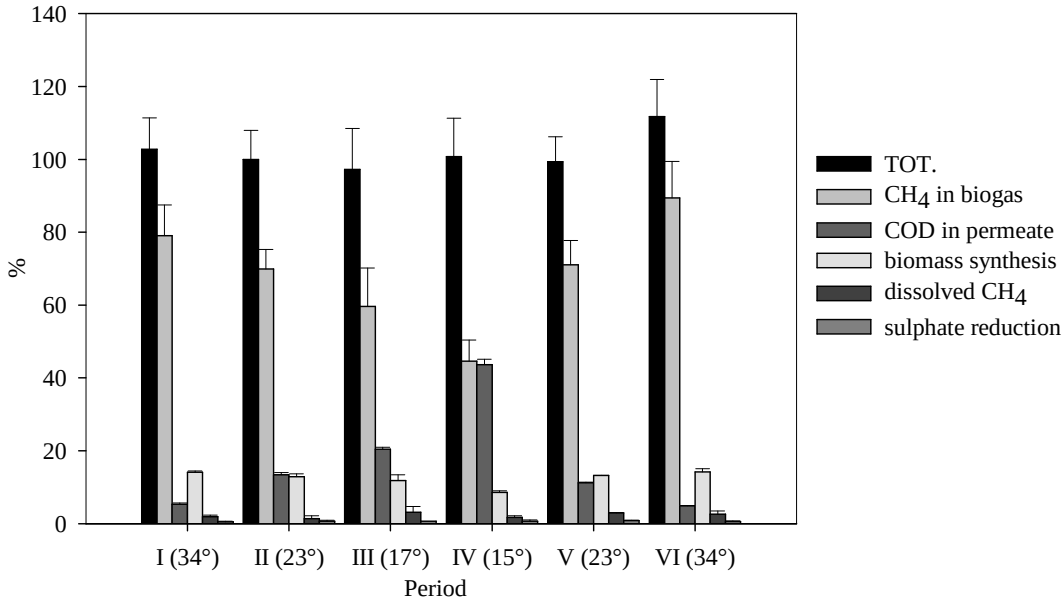


Fig. 3. Temporal variations of different monitored parameters when operating at 15°C: **a)** COD mass balance; **b)** Acetic, propionic, isobutyric and n-butyric acid concentrations. (Temporal variations of COD mass balance and VFAs for the entire experiment duration are reported in Figure S3 a and b)



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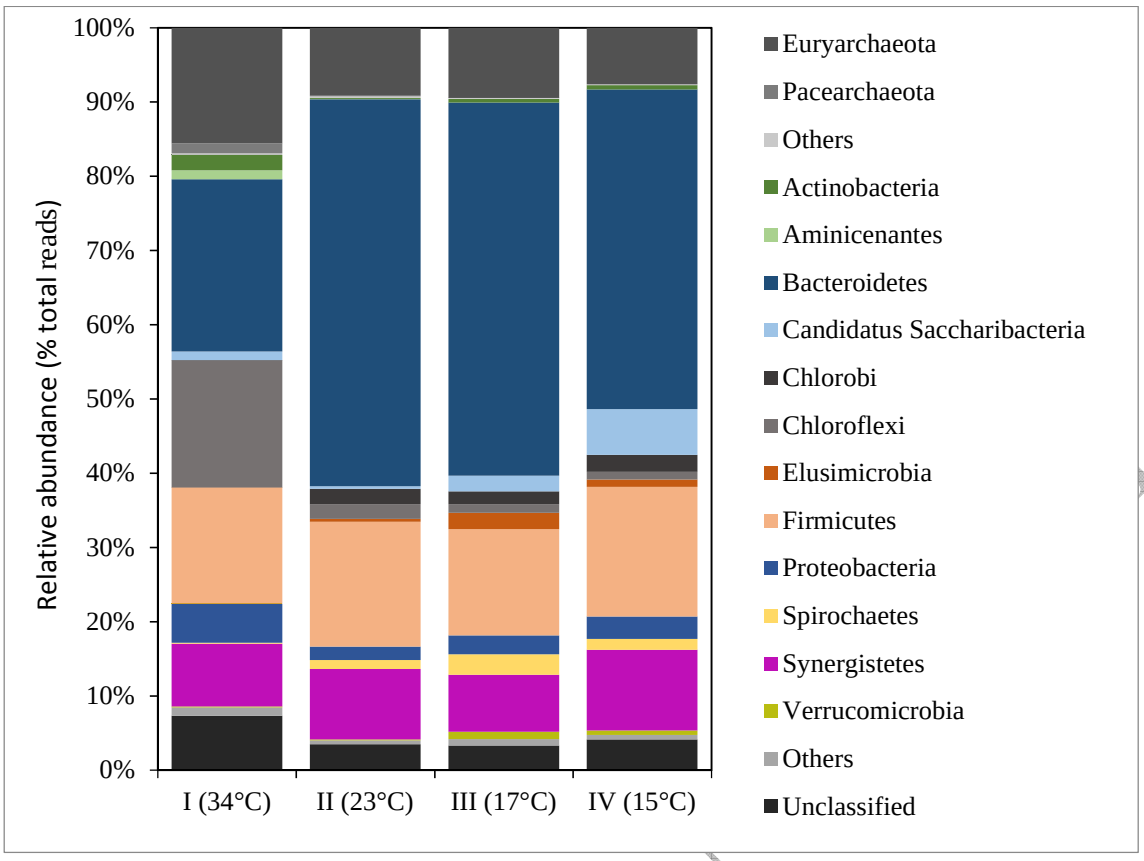

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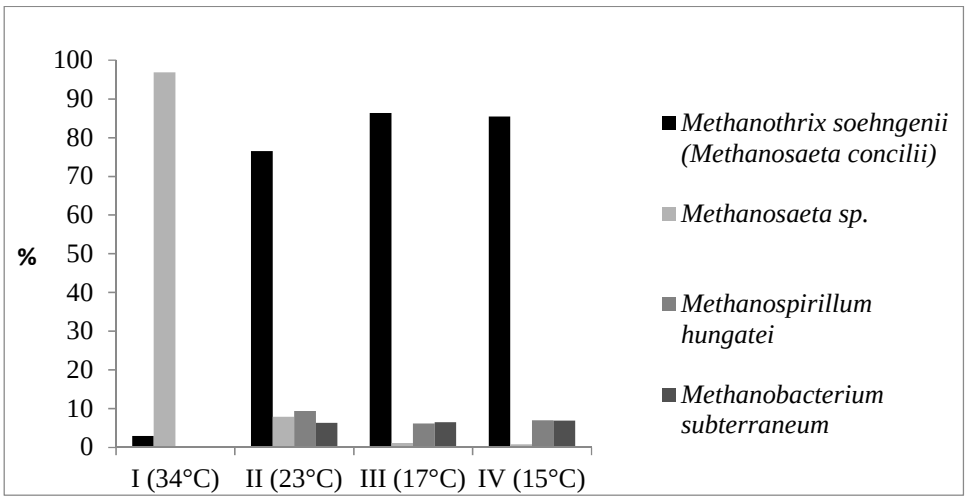

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