



Article Coupling AnMBR, Primary Settling and Anaerobic Digestion to Improve Carbon Fate When Treating Sulfate-Rich Wastewater

Oscar Mateo¹, Pau Sanchis-Perucho², Juan B. Giménez², Ángel Robles², Nuria Martí^{2,*}, Joaquín Serralta¹ and Aurora Seco²

- ¹ CALAGUA—Unidad Mixta UV-UPV, Institut Universitari d'Investigació d'Enginyeria de l'Aigua i Medi Ambient—IIAMA, Universitat Politècnica de Valencia, Camí de Vera s/n, 46022 Valencia, Spain; osmallo@cam.upv.es (O.M.); jserralt@hma.upv.es (J.S.)
- ² CALAGUA—Unidad Mixta UV-UPV, Departament d'Enginyeria Química, Universitat de València, Avinguda de la Universitat s/n, Burjassot, 46100 Valencia, Spain; pau.sanchis-perucho@uv.es (P.S.-P.); juan.b.gimenez@uv.es (J.B.G.); angel.robles@uv.es (Á.R.); aurora.seco@uv.es (A.S.)
- Correspondence: nuria.marti@uv.es

Abstract: The present work involved an assessment of the technical feasibility of coupling AnMBR, primary settling and anaerobic digestion to treat sulfate-rich wastewater at ambient temperature. The innovative approach used focused on reducing the carbon footprint of wastewater treatment while maximizing the energy recovered from influent organic matter. In this process, primary settling reduces the COD/SO₄-S ratio in the influent of the AnMBR system and completely removes organic matter by sulfate-reducing bacteria (SRB), while increasing the COD/SO₄-S ratio in the sidestream anaerobic digester (AD), enhancing energy recovery and biogas quality. This approach has the significant advantage of only producing methane in the AD, so that the AnMBR produces a high-quality, methane-free effluent with no environmental impact from fugitive methane emissions. The performance of this treatment scheme was assessed by operating a demonstration-scale AnMBR plant fed by primary settled municipal wastewater at the hydraulic retention times of 25, 12 and 8.5 h. The results showed that the COD and BOD removed by SRB enabled setting the discharge limits at 25 and 12 h and lowered the carbon footprint to levels below those of an AnMBR plant fed by raw municipal wastewater, mainly by eliminating fugitive methane emissions.

Keywords: digestion; AnMBR; carbon fate; dissolved methane; sulfate-rich wastewaters

1. Introduction

The development of self-sufficient cradle-to-cradle bio-based economic models based on the circular economy (CE) is now being promoted as an alternative to the unsustainable models based on extracting non-renewable raw materials. This shift has been driven by the need to close production cycles and enhance resource sustainability [1]. The CE concept has gained special attention in the wastewater treatment sector [2]. In light of this new CE paradigm, wastewater is no longer seen as waste to be disposed of, but rather as a valuable resource that contains useful components such as clean water, energy and nutrients [3]. In this new approach, wastewater treatment plants (WWTPs) are now rather conceived as water resource recovery facilities (WRRFs).

Anaerobic technology is gaining prominence as the core technology of the newly conceived sustainable WRFFs, with the significant advantage of acting as net energy producers, since no external energy input is required for aeration, and a fraction of the energy contained in the organic matter can be recovered as methane. Anaerobic treatment also enables nutrient recovery, since organic nutrients undergo mineralization, while anaerobic microorganisms produce lower biomass yields than aerobic microorganisms, so that there is less excess sludge to be handled [4].



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). However, anaerobic treatments may also have limitations under certain operating conditions (e.g., treatment flow rate, temperature, etc.) and when treating particular types of waste (e.g., low-strength wastewaters, such as urban wastewaters), mainly due to the low biomass growth rate at sub-mesophilic temperatures and their sensitivity to process dynamics. Anaerobic processes have been traditionally used for high-load streams such as industrial wastewaters and urban WWTP sludge. These high-loaded streams produce large amounts of biogas that can be used to heat the digester to mesophilic or thermophilic levels. According to [5], mesophilic anaerobic processes are economically feasible when influent COD exceeds 4–5 g COD·L⁻¹. Municipal wastewater (MWW) is usually produced at high flow rates and features a low organic load (typical COD levels below 1 g L⁻¹). MWW anaerobic treatment is thus only feasible in warm climates, since it requires large reactor volumes and a great deal of energy to heat the digester to mesophilic conditions.

Anaerobic membrane bioreactors (AnMBRs) offer a promising solution to addressing the challenges of applying anaerobic processes to low-loaded wastewaters, including municipal wastewater (MWW). This innovative technology combines an anaerobic reactor and a membrane filtration system to completely retain the slow-growth microorganisms and its advantages are increasingly attracting the attention of the scientific community [4,6].

The concentration of influent sulfate (SO_4^{2-}) is a key factor in the performance of anaerobic reactors. As sulfate-reducing bacteria (SRB) can be used in a wide range of substrate utilization processes, this leads to competition with the microorganisms involved in anaerobic digestion. They can compete with methanogens, acetogens or fermentative microorganisms for the available acetate, H₂, propionate and butyrate in anaerobic systems [7]. SRB proliferation is considered detrimental to the energy balance due to the production of H_2S instead of methane. SRB consume 2 kg of COD to reduce 1 kg of influent SO_4 -S. From a thermodynamic point of view, SRBs should outcompete the methanogenic consortia during growth on these substrates, so that sulfate reduction would theoretically become the dominant process in anaerobic digesters treating sulfate-rich wastewater [8]. SRB use sulfate as the electron acceptor, resulting in the formation of hydrogen sulfide (H₂S) as the main product. This compound also causes corrosion problems and acts as an inhibitor for both methanogens and SRB. The inhibitory effect of hydrogen sulfide depends on the pH value involved, since the dissociation of this compound is pH-dependent. Between 20% and 50% of the sulfide is present in its unionized form (H_2S) at a pH close to neutrality (pH = 7-7.6) and a temperature of 20 °C.

Several examples can be found in the literature that describe the AnMBR systems applied to treating sulfate-rich wastewaters. According to [9], 19% of influent COD was converted into methane at 27 °C, with an influent COD/SO_4^{-2} ratio of 4.2. The study described in [10] reported on the performance of an AnMBR operated for more than 600 days at ambient temperature and fed with sulfate-rich high-loaded MWW. The percentage of COD converted into methane in this case varied between 20% and 48%, primarily influenced by the influent characterization and temperature. In all these instances, sulfate reduction prevented higher levels of methane production. The percentage of COD consumed by SRB varied between 20 and 50%, while the methane dissolved in the permeate accounted for 5–10% of the influent COD. Since methane is a greenhouse gas with a global warming potential equivalent to 25–34 kg CO_2 .kg CH_4^{-1} , dissolved methane recovery is required to reduce greenhouse gas emissions. The influent SO_4^{2-} concentration has been shown to be a key issue in the economic feasibility of applying AnMBRs to MWW [3].

A treatment scheme based on the combination of an AnMBR with conventional treatments (i.e., primary settler (PS) + AnMBR + AD) has been proposed to mitigate the adverse effects of high influent SO_4^{2-} concentrations [11], compared by simulations of the performance of the AnMBR and a combination of AnMBR and conventional treatments (i.e., primary settler (PS) + AnMBR + AD) for different operating scenarios: sulfate-rich and low-sulfate MWW treatment.

In this context, this paper evaluates the results obtained in a demonstration-scale AnMBR plant in removing organic matter from the effluent of a primary settler of a municipal WWTP using dissimilative sulfate-reducing processes. It also describes the successful results obtained in the meeting of BOD and COD discharge limits by means of SRB. For this, an AnMBR demonstration-scale plant fed by primary-settled municipal WWTP was operated at ambient temperature for 250 days. After validating the technical feasibility of the treatment system, the HRT was reduced to optimize the reactor volume for further full-scale implementation. This paper also discusses the advantages of this treatment scheme over conventional WWTPs and AnMBR systems.

2. Materials and Methods

2.1. Demonstration-Scale AnMBR Plant

This study was mainly carried out by operating a demonstration-scale AnMBR plant consisting of an anaerobic reactor (1.3 m³, working volume of 0.9 m³) coupled with two external membrane tanks (MTs) (0.8 m³, working volume of 0.6 m³ each), giving a total operating volume of 2.1 m³. The MTs were equipped with a commercial hollow-fiber ultrafiltration membrane system (PURON[®] KMS PUR-PSH31, 31 m², 0.03 µm pore size), achieving a total filtration area of 62 m². Two liquid pumps (CompAir; NEMO) continuously pumped the anaerobic reactor content to the MTs at a liquid flow rate of about 2.9 m³ h^{-1} (hydraulic retention time in both MTs of about 12 min), ensuring a proper mixture in the system. The anaerobic sludge was recirculated to the anaerobic reactor from the MTs by overflow, maintaining a constant liquid level in the membrane systems. An additional external liquid pump (CompAir; NEMO) was used to continuously recirculate the contents of the anaerobic reactor at a flow rate of 2.4 m³ h⁻¹ to enhance sludge homogeneity in this element. Filtration was performed by vacuum, using two liquid pumps (JUROP VL02, NBR), one in each MT, to obtain the membrane permeate. Generated permeate was accumulated in an additional clean-in-place (CIP) tank to allow backwashing when necessary. The gas produced in the anaerobic reactor was injected from below the MTs by a blower (FPZ 30HD) to apply gas scouring as the fouling control strategy in the membrane systems. A fraction of this gas was also injected from below the anaerobic reactor to enhance homogeneity. The remaining head space in the anaerobic reactor and MTs was completely interconnected to ensure a constant gas pressure in the system, regardless of the recirculated gas between these elements.

The AnMBR plant was fed with the primary settler supernatant from the full-scale Conca del Carraixet WWTP (Valencia, Spain). A 0.5 mm screen size rotofilter (RF) was employed as pretreatment for this influent to avoid possible large-sized pollutants, such as plastics or fibers, from escaping from the primary settler and reaching the membrane systems. This pretreated wastewater was accumulated in an equalization tank (ET) (0.6 m³) to continuously feed the AnMBR system as required. A liquid pump (CompAir; NEMO) fed the anaerobic reactor with the pretreated influent, maintaining the level of the liquid in the AnMBR system. Further information on the AnMBR plant can be found in [12]. Figure 1 shows a flow diagram of the alternative system proposed in this work, including a plan of the described demonstration-scale AnMBR.

The described demonstration-scale AnMBR plant was equipped with multiple sensors and actuators for its complete automation. Regarding sensors, the anaerobic reactor was fitted with pH-temperature and redox on-line sensors (Endress+Hauser model Liquiline M pH-ORP CM42) to provide continuous information from the process; two pressure sensors were installed at the top and bottom of the anaerobic reactor to control the level of the liquid in the reactor and the gas pressure. Two additional pressure sensors (Endress+Hauser model Cerabar M PMC41) were fitted to the MTs to capture the liquid level and transmembrane pressure (TMP) during filtration. The system pumps (influent, anaerobic reactor and MTs recycling sludge and permeate) were equipped with a complementary flowmeter (Endress+Hauser model Proline Promag 50) to monitor the individual operating liquid flow rates. The gas injected into the MTs and anaerobic reactor was also monitored by three gas flowmeters (Iberfluid model VORTEX 84F) in the individual lines. Regarding actuators, multiple on–off valves were installed in the system to avoid undesirable liquid flows during pumping stops (e.g., during membrane relaxation phases). Frequency controllers (Micromaster Siemens 420; Siemens, Munich, Germany) were connected to the liquid pumps and blowers to accurately control the different fluid flow rates. All the information was captured from the sensors by a programmable logic controller (PLC) designed for the operation of this AnMBR plant, sending all this information to the Supervisory Control and Data Acquisition system (SCADA) system (Simatic WinCC) in a PC. All the automatic actuations were also programmed in the PLC for the complete automation of this AnMBR plant, allowing changes in the corresponding control protocols and set points through the SCADA software. Further information on the automation and control protocols used in the demonstration-scale AnMBR plant can be found in [13].



Figure 1. Flow diagram of the proposed treatment scheme. Abbreviations: RF, rotofilter; ET, equalization tank; MT, membrane tank; CIP, clean-in-place.

2.2. Influent Wastewater Characterization

The AnMBR was fed with effluent from the primary settler of the full-scale "Conca del Carraixet" WWTP. Data were gathered from previous studies [11,12,14] in which this AnMBR was fed with pretreated wastewater (after grit and grease removal) to compare the performance of the treatment scheme both with and without a primary settler. A comparison of both streams is described in Section 3.1.

2.3. AnMBR Plant Operation and Experimental Design

The AnMBR plant was inoculated with anaerobic sludge from the conventional anaerobic digester of the full-scale WWTP "Conca del Carraixet" (Valencia, Spain). This inoculum was operated at a solid retention time (SRT) of 70 days and a hydraulic retention time (HRT) of around 25 h in order to acclimatize the microorganisms before the experiment. The temperature was not controlled (free and subject to daily and seasonal dynamics) to reduce the process energy demands, with microorganisms also previously acclimatized to these conditions before experimentation. The acclimatizing period lasted for about 70 days and allowed the sulfate-reducing bacteria to fully dominate the anaerobic sludge, displacing the methanogenic archaea. The AnMBR experimental period lasted for 8 months, maintaining the SRT at 70 days while modifying the HRT from 25 h (Period I) to 12 h (Period II) and 8.5 h (Period III) (see Table 1). The influent flow rate was thus increased as the system HRT was reduced to determine the minimum reactor volume required for the process.

Table 1. Operating conditions evaluated in the AnMBR unit for the three experimental periods (I, II, III). Abbreviations: SRT, sludge retention time; HRT, hydraulic retention time; OLR, organic loading rate.

Operating Conditions					
Period	Flow Rate	SRT	HRT	Temperature	OLR
	$L \cdot d^{-1}$	d	h	°C	$g COD \cdot L^{-1} \cdot d^{-1}$
Ι	2032 ± 27	70 ± 7	25.3 ± 4.2	25 ± 1	0.27 ± 0.04
II	4335 ± 43	68 ± 8	12.3 ± 3.4	15 ± 2	0.58 ± 0.07
III	5929 ± 31	69 ± 7	8.5 ± 3.4	15 ± 2	0.79 ± 0.06

Filtration was performed by following reiterative filtration–relaxation (F–R) phases, thus controlling the reversible fouling in the membrane systems. Filtration was set at 250 s while relaxation was set at 50 s. Other membrane phases were also performed to minimize membrane fouling, them being backwashing, ventilation and degasification. Backwashing was performed with the permeate generated and accumulated in the CIP tank every 10 F–R cycles at a time lapse of 30 s; ventilation occurred every 10 F–R cycles at a time lapse of 30 s. Ventilation and degasification phases were performed to reduce gas drag/absorption in the pumped permeate, thus reducing cavitation-related issues and gas emissions. Further information on the different stages and their interactions during the AnMBR operations can be found in [11].

Throughout the experimental period, the filtration phases remained unchanged, and only one module was operated at a time, so that net transmembrane fluxes of about 2.68, 5.51 and 7.92 L h⁻¹ m⁻² were achieved for the HRTs considered in this work: 25.3, 12.3 and 8.5 h, respectively. These operating transmembrane fluxes were significantly lower than those expected in industrial applications. It is important to note that this study did not aim to evaluate the filtration performance of the system itself; instead, these low transmembrane fluxes were deliberately used to avoid membrane fouling issues. To further address this concern, gas sparging with the biogas produced was also used at a specific gas demand of 0.23 Nm³ h⁻¹ m⁻² for each MT, so that membrane chemical cleaning was not required during the entire experimental period.

2.4. Analytical Methods

Samples were collected three times a week from the influent, effluent and anaerobic sludge to evaluate the performance of the biological process performance.

Total suspended solids (TSSs), volatile suspended solids (VSSs), total and soluble chemical oxygen demand (COD_T and COD_s, respectively), total and soluble biological oxygen demand at 20 days (BOD_T and BOD_S, respectively), volatile fatty acids (VFAs), alkalinity (Alk), sulfate (SO₄-S), sulfide (S^{2–}-S), nutrients (NH₄-N and PO₄-P), total nitrogen (TN), filtered total nitrogen (TN_f), total phosphorous (TP) and filtered total phosphorous (TP_f) were analyzed in the influent and effluent streams. Total suspended solids (TSSs), volatile suspended solids (VSSs), mixed liquor suspended solids (MLTSs), mixed liquor volatile suspended solids (MLVSs), COD_T, BOD_T, TN and TP were analyzed in the anaerobic sludge.

The analyses were carried out according to standard methods [15], except for the volatile fatty acids (VFAs) and alkalinity (Alk), which were measured by titration following the method proposed by the South African Water Research Commission [16]. Total nitrogen was measured by commercial standard kits (ISO 11905-1; Merck, Darmstadt, Germany). BOD_T and BOD_S were measured by the experimental method based on the Warburg respirometer using the OxiTop WTW experimental design.

The presence of *Escherichia coli* and other coliform pathogens in the permeate was quantitatively determined through the positive β -glucuronidase assay using membrane filters, following the UNE-EN ISO 9308-1:2014 standard method.

2.4.1. Biogas Composition and Dissolved Methane

The methane fraction of the biogas was measured once a week using a gas chromatograph equipped with a flame Ionization detector (GC-FID; Thermo Fisher Scientific, Waltham, MA, USA). A total of 1 mL of biogas was collected by a gas-tight syringe and injected into a 15 m × 0.53 mm × 1 µm TRACER column (Teknokroma, Barcelona, Spain) which was maintained at 40 °C. The carrier gas was helium at a flow rate of 40 mL·min⁻¹. CH₄ pure gas (99.9995%) was used as the standard.

The concentration of dissolved methane was determined by gas chromatography and the static head space analysis technique described in [12].

2.4.2. Anaerobic Biodegradability and Digestibility Tests

The methane potential of the different samples was assessed by the volumetric method. The automatic methane potential test system (AMPTS[®] II; Bioprocess Control) was used to evaluate influent wastewater anaerobic biodegradability by means of the biomethane potential test, and the AnMBR digestate residual digestibility, by means of the residual biomethane potential test. All the assays were carried out at a constant temperature of 35 °C without nutrient addition. To obtain both the anaerobic biodegradability of the influent wastewater and the digestate residual digestibility, the organic matter converted into methane in the experiments was estimated assuming that 350 STP mL of methane were theoretically produced from 1 g of COD.

The organic matter degraded by SRB in the tests was estimated assuming that the SRB outcompeted the methanogenic consortia and SO₄-S was fully consumed, and 2 kg of COD were consumed by the SRB to reduce 1 kg of SO₄–S. For further information on anaerobic biodegradability see [9].

2.4.3. Carbon Footprint and Energy Calculations

The carbon footprint of the proposed system compared to a mainline AnMBR was estimated considering only the methane emissions from the generated permeate. Methane concentrations in the permeate were calculated considering saturation at the system's operating temperature range (25–15 °C). The methane percentage in the generated biogas was assumed as 65% in both a mainline AnMBR and the AD considered in the present study. The environmental impact of methane emissions was calculated according to the greenhouse gas emissions ratio expressed in the EcoInvent database, which established that 1 kg of methane is equivalent to 29 kg of CO₂. Potential energy recovery from the biomass generated in the AnMBR system and fed to the AD was estimated considering the measured digestibility of this biomass. A theoretical methane yield of 0.35 m³ of methane per kg of COD, a methane calorific power of 9.13 kWh per m³ of methane and a methane CHP system electricity generation efficiency of 35% were considered.

3. Results and Discussion

3.1. Impact of Wastewater Clarification on AnMBR Influent Features

This section explores the effects of diverting a fraction of the influent biodegradable COD to be treated in a downstream anaerobic digestion step in a primary settler instead of treating the whole amount in an AnMBR. Table 2 compares the average influent composition (mean and standard deviation) of both pretreated and settled wastewater.

It is worth mentioning the high sulfate concentration present in the wastewater, which was a typical value in the geographical area in which the present study was carried out (see [12,17]). The high sulfate concentration in the drinking water sources that supply the area around the WWTP catchment area mainly accounts for the final concentration in the

wastewater. The sulfate concentration remains fairly constant in the drinking water, as shown by the low coefficient of variance.

Demonstration	Mean \pm SD			
Parameter —	Pretreated WW	Settled WW *		
$\frac{1}{\text{TSS}(\text{mg}\cdot\text{L}^{-1})}$	315 ± 171	97 ± 10		
VSS (%)	80.4 ± 8.0	78.4 ± 5.3		
$COD_T (mg COD \cdot L^{-1})$	591 ± 249	279 ± 39		
$COD_S (mg COD \cdot L^{-1})$	84.0 ± 22.2	98 ± 17		
$BOD_T (mg BOD \cdot L^{-1})$	390 ± 138	170 ± 14		
$BOD_S (mg BOD \cdot L^{-1})$	66.5 ± 33.0	50 ± 9		
VFA (mg COD·L ^{-1})	7.9 ± 10.2	13.4 ± 6.6		
Alkalinity (mg CaCO3·L ^{-1})	338 ± 65	486.7 ± 59.0		
SO_4 -S (mg S·L ⁻¹)	101.7 ± 19.9	97.4 ± 10.3		
COD_T/SO_4 -S (mg COD·mg ⁻¹ S)	6.0 ± 2.9	2.6 ± 0.2		
TN (mg N·L ^{-1})	55.3 ± 12.2	48.9 ± 9.7		
TP (mg $P \cdot L^{-1}$)	10.1 ± 3.5	5.5 ± 0.4		
NH_4 -N (mgN·L ⁻¹)	33.1 ± 9.1	41.8 ± 7.5		
PO_4 -P (mg P·L ⁻¹)	4.1 ± 1.6	3.5 ± 0.6		
Anaerobic biodegradability (%)	68.5 ± 2.8	64.3 ± 1.8		
Aerobic biodegradability (%)	63.1 ± 8.9	60.5 ± 1.3		

Table 2. Average AnMBR influent composition.

Note: * Settled WW refers to influent quality fed to the AnMBR system after rotofilter pretreatment.

In the configuration without a primary settler, the pretreated wastewater was directly fed into the AnMBR. A total of 52.9% of the biodegradable COD was consumed by SRB, according to the influent COD/SO₄-S average ratio, while the remaining biodegradable COD (47.1%) was available to the methanogenic organisms. On the other hand, methane was produced in the liquid phase and further partitioned between the liquid and gas phases. Assuming that the liquid–gas phase equilibrium was achieved as a result of the biogas-assisted mixing in the AnMBR [12], the CH₄ saturation concentration in the liquid phase, and hence leaving the system as dissolved methane, depended on the operating temperature, accounting for 18.1%, 15.1% and 13.2% of the influent biodegradable COD at operating temperatures of 15 °C, 25 °C and 33 °C, respectively.

Alternatively, to simulate introducing a primary settler into the treatment scheme, the AnMBR feed was shifted to the settled wastewater from the full-scale WWTP primary settler effluent. In this configuration, a fraction of the organic load which was previously fed to the AnMBR (i.e., the difference in the organic load between the pretreated wastewater and the settled wastewater) would be diverted to a conventional anaerobic digester, while most of the soluble components and the non-settling fractions of the suspended matter continued to be fed to the AnMBR. The efficiency of the full-scale WWTP primary settler in removing the suspended biodegradable COD was 62.9%, achieving a total biodegradable COD removal of 56.4%. The soluble and non-settling biodegradable COD fraction, representing the remaining 43.6%, continued to be fed to the AnMBR. Under these conditions, the COD consumed by the SRB virtually accounted for the total BOD entering the AnMBR, leaving no chance for the methanogenic organisms to grow. As a result, there was no methane production in any of the periods in which the AnMBR was fed with the settled wastewater, thus preventing any fugitive methane emissions. SRB were thus responsible for consuming the biodegradable COD and allowed the system to meet the discharge limits at an HRT of 12 h or higher. It should be noted that the settled wastewater's biodegradable COD/SO₄-S ratio was lower than 2, indicating that there was insufficient substrate for the SRB to complete the dissimilative reduction of all the sulfate, as shown by the presence of sulfate in the effluent (more information in Section 3.2.3).

The primary settler plays a crucial role in concentrating the suspended organic matter in the influent, resulting in a primary sludge stream with a higher biodegradable COD/SO₄-S ratio (sulfate solubility prevents it from concentrating in the primary settler), which is to be diverted to an anaerobic digester. The higher the organic matter concentration in the primary settler, the lower the primary sludge flow rate, which eventually determines the COD/SO₄-S ratio. The higher COD/SO₄-S ratio in the digester enables the methanogens to outcompete the SRB, so that they use up most of the available biodegradable COD required to produce methane. Also, the lower flow rate to the anaerobic digester reduces the dissolved methane and fugitive methane emissions.

Figure 2 shows the predicted distribution of the biodegradable COD in the primary sludge fed to the downstream anaerobic digestion step, calculated under the following assumptions:

- Complete dissimilatory sulfate reduction takes place.
- The biodegradable COD left from dissimilatory sulfate reduction is available for MA.
- Methane partition between liquid and gas phases takes place according to the equilibrium.



Figure 2. Forecast of the distribution of the biodegradable COD entering the anaerobic digestion as a function of the primary sludge BOD concentration. Abbreviations: D-CH₄, dissolved methane; BG-CH₄, methane in the biogas.

The primary sludge concentration and flow rate were calculated according to the primary settler mass balances. The descending BOD fraction being diverted to SRB as the BOD concentration in the primary sludge increased highlights the soluble nature of sulfate and the competitive advantage of SRB over MA and thus, the higher the concentration of BOD in the primary sludge, the lower the flow rate and the sulfate load entering the anaerobic digestion step. The biodegradable COD available to MA thus increases as the primary sludge concentration rises. Considering an adequate mass transfer in the digester, it can be assumed that methane was saturated in the liquid phase. According to Henry's Law, the dissolved methane concentration was thus constant, regardless of the primary sludge BOD concentration and the fugitive methane emissions decreased as the primary sludge flow rate dropped, i.e., as the concentration rose.

Figure 3 shows the predicted distribution of the influent biodegradable COD in both the AnMBR alone at an operating temperature of 33 °C and the alternative treatment approach proposed in this work, consisting of a primary settler, an AnMBR and a conventional anaerobic digester (PS + AnMBR + AD), assuming a BOD concentration for the primary sludge of 10 g·L⁻¹. As already mentioned, in this alternative configuration, 43.6% of the influent BOD continued to be fed to the AnMBR and was consumed exclusively by the SRB, whereas the remaining 56.4% was settled and concentrated in a primary settler. The concentrated stream was then diverted to a conventional anaerobic digester where, for a primary sludge concentration of 10 gBOD·L⁻¹, only 2% was consumed by the SRB, representing 1.1% of the influent's biodegradable COD. Therefore, the biodegradable COD consumption via dissimilatory sulfate reduction in the alternative configuration accounted for 44.7 % (43.6% in the AnMBR + 1.1% in the AD). The remaining 98% of the biodegradable COD diverted to the downstream AD was available for methanogens to produce methane.



% SRB-AnD

% SRB-AnMBR

An amount of 0.5% of the methane produced remained in the liquid fraction as dissolved methane, accounting for 0.3% of the influent biodegradable COD (see Figure 2).

Figure 3. Availability of influent biodegradable COD in the treatment scheme, including a primary settler, for a primary sludge BOD concentration of 10 g·L⁻¹.

Including a primary settler therefore enables the redistribution of biodegradable COD in an influent stream. Firstly, the lower biodegradable COD/SO₄-S in the effluent of the primary settler reduced the biodegradable COD consumption by SRB from 52.9% to 43.6% in the AnMBR, since there was not enough substrate for the SRB to complete dissimilatory sulfate reduction. Secondly, the concentration of the suspended biodegradable COD in the primary sludge stream resulted in a high biodegradable COD/SO₄-S ratio, which allowed methanogens to outcompete the SRB in the downstream anaerobic digestion step. The biodegradable COD consumed by the SRB was 1.1% of the influent load, resulting in an overall biodegradable COD available for dissimilative sulfate reduction in the (PS + AnMBR + AD) of 44.7%, which is 8.2% lower than treatment in an AnMBR alone.

The remaining 55.3% of the influent biodegradable COD was available for the methanogens in the anaerobic digester, from which as little as 0.3% ended up as dissolved methane under mesophilic conditions. This represents a reduction of 97.7% of the dissolved methane in the AnMBR process under the most favorable operational conditions (i.e., 33 $^{\circ}$ C).

Finally, the percentage of the influent biodegradable COD ending up as methane in the biogas in the (PS + AnMBR + AD) accounted for 55%, i.e., 61.8% higher than the treatment in an AnMBR alone under the most favorable operational conditions (i.e., 33 $^{\circ}$ C).

3.2. AnMBR Performance

□% SRB

🔳 % D-CH4 🛛 📕 % BG-CH4

3.2.1. COD and TS Evolution

The demonstration plant was operated for more than 250 days at ambient temperature. The temperature varied between 32 and 12 °C during this period (see Figure 4) and the influent flow rate was increased from 2032 to 5929 $L \cdot d^{-1}$ to evaluate the impact of HRT on treatment performance. After reaching a pseudo steady state, the AnMBR performance was thoroughly characterized. The influent flow rate was then gradually increased until reaching the value established for the next operational period (see Table 1).

Figure 4 shows the changes in the MLTS, MLVS, temperature and COD concentrations in the AnMBR during the operational periods. The data used to calculate the AnMBR performance in the pseudo steady states achieved are indicated by vertical lines. Pseudo steady states were considered to be reached when the MLTS, MLVS and effluent composition showed no significant variations (less than 10%).

As can be seen in Figure 4, at the beginning of the operational period, the MLTS concentration continuously declined due to variations in the operating conditions, as the demonstration plant had previously been fed with raw wastewater. The rise in MLTSs from day 87 to day 95 can be attributed to replacing the blower and improving the sludge mixing. Figure 4 also shows that MLTS concentration increased from values of around 3000 mg· L⁻¹ in the first period (T = 25 °C, HRT = 25 h) to values of over 8000 mg·L⁻¹ in the third (T = 15 °C, HRT = 8.55 h). The significant increase in MLTS concentration was due to both

the higher influent flow rate and the lower temperature. Lower temperatures produced lower hydrolysis rates and reduced the biodegraded COD. This is also evident from the substantial increase in the percentage of volatile solids between the first and second periods. The higher organic loading rate between Periods II and III significantly increased MLTS concentration, although the percentage of MLVSs remained constant because of the similar temperature in both periods. It should be noted that MLTS concentrations were lower than the typical values (12–15 g·L⁻¹), since the demonstration plant was originally designed to deal with raw wastewater rather than primary settled wastewater.



Figure 4. Changes in MLTS, MLVS, temperature and COD concentrations in the AnMBR during the three periods.

Effluent VFA was used as the indicator to assess the biomass's adaptation to the variations in organic loading rates during the operational periods. The effluent VFA concentration remained negligible (below 5 mg L^{-1}) during all three steady states. There were only slight increases (around 20–30 mg· L^{-1}) at the beginning of the second and third periods. These modest variations in effluent VFA concentrations indicate that the biomass adapted well to the changes in organic loading rates and that the AnMBR system's performance remained effective and stable during all the periods.

3.2.2. Waste Sludge Production and Characterization

Table 3 gives the sludge characterization data including the concentration of COD, MLTS, MLVS, the aerobic and anaerobic digestibility and the sludge production in all three pseudo steady states.

In the first period, the high SRT and temperature helped to enhance hydrolysis of particulate organic matter and produced the lowest percentage of MLVSs (54%) and sludge production of 101 g VSS·kg CODremoved⁻¹ and 79 g VSS·kg CODinf⁻¹.

HRT declined in the second period, along with a significant drop in temperature (10 °C between the pseudo steady states of both periods), significantly raising the percentage of MLVSs (66% in the second and third periods). The considerable increase in MLTSs (due to lower HRT and temperature) raised sludge production. This increase is significantly higher when sludge production is expressed as g VSS·kg COD removed⁻¹ since the amount of COD removed decreased during the experimental period (see the following section). The sludge production obtained in this study is significantly lower than the values obtained

in previous experiments (218–370 g VSS·kg COD removed⁻¹) [9], which can be attributed to the fact that our pilot plant was fed with settled wastewater instead of pretreated wastewater.

	Mean \pm SD		
Parameter	Period I	Period II	Period III
$COD_{sludge} (mg COD \cdot L^{-1})$	2783 ± 172	5339 ± 182	8719 ± 147
MLTS (mg·L ^{-1})	2941 ± 182	5061 ± 232	8061 ± 247
MLVS (mg·L ^{-1})	1603 ± 172	3349 ± 231	5349 ± 236
MLVS (%)	54 ± 2	66 ± 2	66 ± 2
Sludge production (g VSS·kg ⁻¹ COD _{inf})	79 ± 6	91 ± 8	96 ± 9
Sludge production (g VSS·kg ⁻¹ COD _{rem})	101 ± 8	124 ± 9	176 ± 16
Aerobic sludge digestibility (%) ^a	49.0 ± 1.4	57 ± 1.2	59 ± 1.3
Anaerobic sludge digestibility (%) ^b	9.8 ± 1.2	25.0 ± 0.7	34.0 ± 0.7

Table 3. Sludge characterization and production in each experimental period.

Notes: ^a Calculated as the sludge BOD_T/COD ratio. ^b Measured in anaerobic assays carried out in bioprocessing.

Sludge digestibility is related to the degree of sludge stabilization. The very low value of anaerobic digestibility (9.8 \pm 1.2%) obtained for the first period (SRT = 70 d, HRT = 25 h, T = 25 °C) indicates that almost all the biodegradable organic matter was degraded by SRB. In fact, this value is similar to that obtained by [9] at SRT = 140 d, T = 27 °C and HRT = 24.4 h. This result demonstrates that an SRT = 70 days and HRT of 25 h provide sufficient time to remove all biodegradable organic matter, rendering further biological treatment unnecessary. As the HRT and temperature decreased, the amount of organic matter degraded by SRB also decreased and raised anaerobic digestibility to 25% at HRT = 12 h and 34% at HRT = 8.5 h. This can be explained by the non-degraded soluble organic compounds, since the percentage of MLVSs is the same in both periods. Feeding this sludge to the anaerobic digester thus would boost methane production.

On the other hand, the aerobic digestibility results of all periods are considerably higher (49%, 57% and 59%) than their respective anaerobic digestibility (10%, 25% and 34%). These results suggest the presence of organic compounds in the AnMBR sludge that could not be converted into methane but could be degraded aerobically. These values agree with those reported in previous studies [9].

3.2.3. Effluent Characterization

The effluent of the AnMBR pilot plant showed negligible suspended solid concentrations during the whole experimental period. As can be seen in Figure 5a,b, effluent COD and BOD₅ increased as the HRT was reduced. Effluent COD and BOD₅ concentrations rose slightly from Period I to Period II, despite the significant drops in HRT (from 25 to 12 h) and temperature (from 25 °C to 15 °C). However, the increase in these parameters between Periods II and III was much more substantial. In the third, the European discharge quality standards (European Wastewater Directive, CE 91/271) for these parameters were not met (see dashed red line in Figure 5a,b). The reduced HRT in Period III limited the fermentation of part of the soluble biodegradable organic matter by acidogenic bacteria, which in turn led to less mineralized nutrients. These results indicate that acidogenesis was incomplete when the demonstration plant was operated at 15 °C and an HRT of 8.5 h. It should be noted that effluent VFA concentration was negligible even in Period III. All the VFAs produced by acidogenic bacteria were consumed by SRB.

Table 4 shows the influent sulfate concentration and effluent sulfate and sulfide concentrations. As can be seen in Table 4, in Period I, nearly all the sulfate was consumed by SRB, indicating efficient sulfate reduction. However, in Periods II and III, VFA production was not enough to completely remove all the sulfate. In fact, soluble COD in the permeate (127 mg COD·L⁻¹) was higher than in the influent wastewater in Period III (98 mg COD·L⁻¹), implying that the amount of hydrolyzed organic matter was higher than that of fermented organic matter. The limiting step in this period was the fermentation stage but hydrolysis was also affected by the lower HRT and temperature.



Figure 5. Average effluent composition for (**a**) COD_{ef} and (**b**) BDO_{5ef} . The dash red line indicates the European discharge quality standards (European Wastewater Directive, CE 91/271).

		$\mathbf{Mean} \pm \mathbf{SD}$	
Parameter	Period I	Period II	Period III
SO_4 - S_{inf} (mg S·L ⁻¹)	92.6 ± 8.2	102 ± 7.5	98.5 ± 6.3
SO_4 - S_{ef} (mg S·L ⁻¹)	5.2 ± 1.1	40.9 ± 3 ,2	73.2 ± 6.9
$S^{2-}-S_{ef} (mg S \cdot L^{-1})$	88.9 ± 9.5	66 ± 2.1	37.5 ± 2.6

Table 4. Influent sulfate and effluent sulfate and sulfide concentrations in each experimental period.

Table 5 shows the estimations of the amounts of hydrolyzed and fermented organic matter for the three periods evaluated expressed as daily amounts and milligrams per liter of influent wastewater. The amount of organic matter fermented was estimated from the sulfate removed, considering that 2 g of COD are required to reduce 1 g of SO₄-S. Hydrolyzed organic matter was estimated from fermented organic matter and the difference between the soluble COD in the influent and effluent.

Table 5. Fermented and hydrolyzed COD in each experimental period.

Parameter	Units	Period I	Period II	Period III
Fermented COD	$(mg \cdot L^{-1})$	175	122	51
	$(g \cdot d^{-1})$	355	530	300
Hydrolyzed COD	$(mg L^{-1})$	146	97	93
	$(g \cdot d^{-1})$	296	421	549

As can be seen in Table 5, hydrolysis was significantly affected by the organic loading rate and temperature. The former rose from Period I to Period III due to the lower HRT. The higher the organic loading rate, the higher the amount of hydrolyzed organic matter. However, due to the significant drop in temperature between Periods I and II, the concentration of hydrolyzed influent organic matter fell from 146 mgCOD·L⁻¹ to 97 mgCOD·L⁻¹. HRT did not affect the hydrolysis rate since this concentration was similar in Periods II and III.

The fermentation rate was significantly affected by temperature, organic loading rate and HRT. From Periods I to II, the total amount of fermented organic matter increased due to the significant increase in the organic loading rate, but the percentage of influent organic matter decreased due to the low temperature values in this period. Both the total amount of fermented organic matter and the percentage of fermented influent COD fell between Periods I and III due to the low HRT value (8.5 h), which was too low for the fermentative bacteria to maintain their activity. The effect of HRT and temperature on the hydrolysis rate can also be seen in the mineralization of organic nitrogen. The differences between influent and effluent ammonium concentrations were 5.2 mg NH₄-N·L⁻¹, 3.5 mg NH₄-N·L⁻¹ and 3.2 mg NH₄-N·L⁻¹ for Periods I, II and III, respectively. The lower the hydrolysis rate, the smaller the increase found in the effluent ammonium concentration. The ratio of the increased ammonium concentration and the amount of hydrolyzed organic matter was similar in all three periods, with values of 0.035, 0.036 and 0.034 g N/g COD, respectively, as was found for phosphate concentrations.

3.2.4. COD Mass Balance

Table 6 gives the percentages of COD removed (COD_{rem}) and COD biodegraded (COD_{deg}) for all three periods. The difference between COD_{rem} and COD_{deg} was due to the organic matter withdrawn with the waste sludge. As can be seen in this table, the lower the HRT, the lower the percentage of COD removed and degraded. SRB were responsible for the organic matter degradation in all three periods, since there were no methanogens to generate methane. The percentage of degraded COD in the first period was similar to the measured influent wastewater anaerobic biodegradability (see Table 2), indicating that the high SRT and HRT degraded almost all the biodegradable organic matter in the AnMBR pilot plant. However, as mentioned above, the drop in HRT and temperature from Period I to Periods II and III significantly reduced the growth of acidogenic bacteria, so that SRB growth was limited because neither they nor the acidogenic bacteria were able to efficiently capture hydrolyzed organics.

Table 6. Percentages of COD removed and COD degraded during the different experimental periods.

D ($\mathbf{Mean} \pm \mathbf{SD}$	
Parameter	Period I	Period II	Period III
COD _{rem} (%)	80 ± 2	70 ± 2	56 ± 2
COD _{deg} (%)	64 ± 1	55 ± 1	40 ± 1

Figure 6 shows the COD mass balance for the three periods evaluated. As can be seen, the percentage of waste sludge COD is similar during the entire experimental period because the SRT was maintained constant at 70 days. The main differences between the periods were the percentages of influent COD present in the permeate and degraded by SRB, which were significantly affected by HRT and temperature. In fact, 44% of the influent COD was present in the permeate and the effluent COD and BOD concentrations were above the European discharge limits (see Figure 5).



Figure 6. AnMBR COD balance at each HRT tested: (a) 25 h, (b) 12 h and (c) 8.5 h.

3.3. Potential Benefits of the Proposed Alternative

AnMBR technology is an interesting option when the goal is to enhance resource recovery in municipal wastewater treatment. In fact, numerous studies have reported promising results on recycled water quality and energy savings (see for instance [10]). However, methane emissions from AnMBR permeate, which can reach up to 80% of the methane produced when operating at relatively low temperatures (around 25–15 °C) [18–20], are still an important issue that needs to be solved if this technology is to be implemented in full-scale

WWTPs. Additionally, the treatment of sulfate-rich wastewaters is a serious drawback for this technology, since a substantial fraction of the influent organic matter is consumed by SRB instead of methanogens [21], hindering the energy balance of the process while contaminating the biogas produced with hydrogen sulfide. In this scenario, the proposed treatment scheme therefore appears as an attractive alternative as it would solve both of the above-mentioned issues without losing the potential benefits of AnMBR systems.

The configuration used can sharply reduce the soluble methane emissions from anaerobic effluents, since the mainline AnMBR permeate is completely free of this gas and the liquid effluent produced by the sidestream AD is much smaller and easier to control in this regard. In fact, considering that the classic mainline AnMBR permeate was not treated to reduce the methane lost (methane-saturated permeate at the operating temperature range of this study), the process's carbon footprint could be reduced by about 0.397 (25 °C)–0.478 (15 °C) kgCO₂-eq per m³ of treated wastewater by applying this alternative scheme. This carbon footprint reduction is still considerable (around 0.123 (25 °C)–0.150 (15 °C) kgCO₂-eq per m³ of treated wastewater) even when considering a permeate methane recovery in a classic mainline AnMBR of about 67% (methane recovery value reported by [9]), showing the potential benefits of this proposed alternative from an environmental point of view.

This proposal can also slightly improve the potential energy recovery of AnMBRs. Unlike the conventional wastewater treatment by activated sludge, a mainline AnMBR can completely cover the process's energy demands, estimating neutral energy demands or even a net energy production of about 0.05–0.6 kWh per m³ of treated wastewater [21]. However, including a primary settling step and avoiding methanogens in the AnMBR system, this energy production could be increased by about 0.03 kWh per m³ of treated wastewater. This increment in the energy recovery can be achieved by preventing competition for organic matter between the SRB and methanogens in the mainline AnMBR. Instead, focusing on the energy production in a sidestream AD, the organic matter consumed by sulfate-reducing bacteria can be easily controlled/optimized in the mainline AnMBR by adjusting the operating SRT and HRT (i.e., not all the influent sulfate is reduced in the AnMBR). This outcome is clearly illustrated by the present results (see Table 4), in which the sulfate concentration was higher in the permeate as the HRT was reduced in the AnMBR system. The AnMBR system considered in this scheme should thus be optimized for the proper treatment of the influent wastewater (i.e., meeting discharge limits) at the lower possible HRT and SRT to reduce both the OM consumed by SRB and the system's volume requirements. Concerning results obtained in this study, this objective was met for an HRT of 12 h (OLR around 0.58 gCOD·L⁻¹·d⁻¹; see Table 1), therefore being the optimum HRT around this value. On the other hand, better operating conditions can be used in the sidestream AD to boost methanogenic activity (e.g., temperature, SRT and HRT, concentration of COD/solids in the influent, etc.), also being able to use a fraction of the organic matter consumed by the sulfate reducers by their degradation in the sidestream AD, which receives insignificant sulfate. Furthermore, a cleaner biogas (richer in methane and poorer in hydrogen sulfide) can be achieved by the proposed alternative compared to a classic mainline AnMBR by avoiding the competition for organic matter between sulfate-reducing bacteria and methanogens in the anaerobic treatment. Instead, all the biogas production is boosted in a sidestream AD, therefore avoiding post-treatments for using this biogas which can be traduced in lower operating costs.

The AnMBR produced permeate that met the European discharge standards (considering no sensible environments) by SRB treatment only under the proper operating conditions (12 h of HRT and 70 d of SRT at a temperature of around 15 °C for the conditions established in this work). This permeate could thus be used for fertigation, when possible, to take advantage of the high-quality permeate produced (free of solids and pathogens due to ultrafiltration), while valorizing soluble nutrients. In this scenario, it would be necessary to previously determine any possible drawbacks in using this effluent (with its dissolved H₂S) on crops. However, it is important to highlight that this issue would also exist in permeates generated by conventional AnMBR systems which also completely reduce influent sulfate. The proposed alternative therefore would not be a disadvantage in this regard. Alternatively, when not able to directly apply this effluent for agricultural purposes, soluble nutrients could be concentrated and recovered from this recycled water in a post-treatment stage, for example by membrane contactors, ion exchangers, microalgae culture and harvesting or osmosis filtration. Other alternatives, such as denitrification via sulfide oxidizers [22] could also be used for treating this type of effluent when no other options are available. On the other hand, the higher nutrient concentrations in the sidestream AD after organic matter mineralization could be used to produce commercial fertilizers. In this regard, ammonium could be recovered via membrane contactors as ammonium sulfate. Alternatively, phosphate could be recovered via chemical precipitation as struvite at the appropriate effluent concentration. Auxiliary membrane systems (such as those cited above) could be used to increase the ion concentration in the permeate before this step. When considering this possible green fertilizer source, additional energy savings could be achieved by the proposed system, estimating them at about 19.3 kWh per kg of reused nitrogen and 2.1 kWh of reused phosphorous [9]. Finally, the completely mineralized sludge produced by the AD could be directly applied for agricultural purposes.

A side effect of only using SRB in an AnMBR is that a significantly lower TS can be expected in the reactor than in classic mainline AnMBR systems. Indeed, AnMBRs usually operate at a TS of around 10–15 g·L⁻¹ (see for instance [10,23,24]), while the described system reached a TS of about 5 g·L⁻¹ at the pseudo optimum HRT of 12 h determined in this work. This lower TS concentration could be associated with lower energy demands of the membrane system since a lower fouling could be expected, this being another additional benefit of the proposed alternative. Since SRB are usually an important part of the biomass filtered in classic mainline AnMBRs, no significant differences between the filterability characteristics of the sludge generated by this proposal could be expected, and thus fouling linked to the operating TS. However, further research will be required to confirm this potential benefit. It should also be highlighted that, although the filtration performance was not properly evaluated in this work, gas sparging was adjusted under equivalent values to those reported in other AnMBR systems (see for instance [25]). No relevant energy demand differences with classic AnMBRs are thus expected concerning this energy input.

The gas generated in the AnMBR system would mainly be composed of CO_2 and N_2 since no CH_4 would be produced. The concentrations of other gases, including H_2S , could also slightly increase, although always limited by a liquid–gas equilibrium. Specifically, for an average concentration of 100 mg $S-SO_4/L$ in the influent wastewater, an H₂S saturation concentration of around 3% would be expected at 35 °C and a pH of 6.5 (See Figure S1). This concentration could be considered as slightly higher than that expected in other AnMBRs, where values of about 1.8–0.1% are usually reported [26]. This could represent a slight drawback for the proposed technology, since specialized equipment would be required to impulse this gas without important corrosion. However, since values of this gas around 1.5–2% have also been reported in classic AnMBR systems [9], no significant issues were expected in this regard. On the other hand, the effect of this gas sparging on membrane fouling control will also require future evaluation, since the bubbles generated by the membrane module diffusor may present changes in shape and stability due to the absence of CH_4 . In this case, no significant issues were observed in this respect during the present study and therefore no expecting important differences regarding classic biogas sparging effects. In addition to the above, a valorization of the produced gas stream after H_2S cleaning could also be proposed due to its expected high CO_2 content. Future studies considering this possibility would also be required. Otherwise, this gas could also be directly discharged to the atmosphere with no environmental/safety impact, with the CO_2 emitted not contributing to global warming since it was produced from biogenic sources.

Finally, this proposal also provides benefits concerning space requirements for its implementation in full-scale applications compared to classic mainline AnMBRs. Thanks to taking advantage of sulfate reducers for wastewater treatment (much faster than methanogens consuming organics), lower aerobic reactor volumes would be required (lower SRTs and HRTs available), facilitating its implementation in medium-sized/small municipal WWTPs. This improvement is especially relevant since the other elements considered in this alternative scheme (i.e., primary settler and AD) are commonly used in already operational municipal WWTPs. Thus, the proposed system would have a lower economic/operational impact than classic mainline AnMBR systems in current facilities, enhancing its viability and acceptability.

The proposed treatment scheme could thus be considered an attractive alternative for treating sulfate-rich wastewaters, with significant benefits over classic mainline AnMBR treatments. However, further studies focused on determining the best operating conditions when considering all the interconnected elements (mainly primary settling, AnMBR and AD) would be necessary to boost resource savings (reclaimed water, energy and nutrients), with them intrinsically related to the influent sulfate. On the other hand, a more complete assessment of its economic/environmental impact (i.e., life cycle cost (LCC) and life cycle assessment (LCA)) will also be necessary to properly determine its viability.

4. Conclusions

The main conclusions that can be drawn from this work can be summarized as the following:

- An innovative treatment scheme combining primary settling, AnMBR technology and anaerobic digestion was successfully evaluated in a demonstration-scale AnMBR plant operating for over 8 months.
- The feasibility of a primary settler in combination with AnMBR technology to remove organic matter from sulfate-rich urban wastewater was also demonstrated. Sulfate-reducing bacteria (SRB) effectively removed organic matter while meeting the European Directive criteria on effluents by using influent sulfate as an electron acceptor. No methane production was observed.
- Temperature had a significant impact on the hydrolysis and fermentation rates, with lower temperatures leading to slower biological processes.
- The low suspended solid concentration in settled wastewater allowed for shorter hydraulic retention times (HRTs) in the AnMBR system compared to the typical values for AnMBR systems fed with pretreated wastewater. Organic matter removal was achieved with an HRT of 12 h at 15 °C. When the HRT was further reduced to 8.5 h, the acidogenic bacteria were affected and the production of volatile fatty acids (VFAs) was insufficient to remove influent organic matter.
- Future research is needed to optimize the operating conditions, including SRT and HRT at different temperatures.
- Life cycle cost and life cycle analysis should also be conducted to quantify the advantages of the proposed treatment scheme under different operating conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15203574/s1. Figure S1. H₂S saturation concentration as a function of pH.

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