Long-term operation of a pilot-scale anaerobic membrane bioreactor (AnMBR) treating high salinity low loaded municipal wastewater in real environment

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Abstract

Long term operation of an anaerobic membrane bioreactor (AnMBR) treating municipal wastewater was investigated in a real seawater intrusion spot in Falconara Marittima (Central Italy) on the Adriatic coastline. Changes in biological conversion and system stability were determined with respect to varying organic loading rate (OLR) and high salinity conditions. At an OLR of 1 $kgCOD \cdot m^{3-1} d^{-1}$, biogas production was around 0.39 ± 0.2 $L \cdot d^{-1}$. The increase of the OLR to 2 $kgCOD \cdot m^{3-1} d^{-1}$ resulted in the increase of biogas production to 2.8 ± 1.5 $L \cdot d^{-1}$ (with 33.6% ± 10.5% of CH_4) with methanol addition and to 4.11 ± 3.1 $L \cdot d^{-1}$ (with 29.7% ± 11.8% of CH_4) with fermented cellulosic sludge addition. COD removal by the AnMBR was 83% ± 1% when the effluent COD concentration was below 100 mg $O_2 \cdot L^{-1}$. The addition of the fermented sludge affected the membrane operation; significant fouling occurred after long-term filtration, where the trans-membrane pressure (TMP) reached up to 500 mbar. Citric acid solution was applied to remove scalants and the TMP reached the initial value. High saline conditions of 1500 $mgCl^{-} \cdot L^{-1}$ adversely affected the biogas production without deteriorating the membrane operation. The treated effluent met the EU quality standards of the D.M. 185/2003 and the new European Commission Resolution for reuse in agriculture.

Keywords: AnMBR; membrane fouling; municipal wastewater; fermentation; salinity; cellulosic sludge

Nomenclature

AD: Anaerobic Digestion

AeMBR: Aerobic Membrane Bioreactor

AnMBR: Anaerobic Membrane Bioreactor

CAS: Conventional Activated Sludge

COD: Chemical Oxygen Demand

DO: Dissolved Oxygen

EC: Electrical Conductivity

EPS: Extracellular Polymeric Substances

HRT: Hydraulic Retention Time

MLSS: Mixed Liquor Suspended Solids

MWT: Municipal Wastewater Treatment

OLR: Organic Loading Rate

ORP: Oxidation Reduction Potential

PE: Population Equivalent

sCOD: Soluble Chemical Oxygen Demand

SMA: Specific Methanogenic Activity

SRT: Sludge Retention Time

SuMBR: Submerged Membrane Bioreactor

tCOD: Total Chemical Oxygen Demand

TKN: Total Kejdahl Nitrogen

TMP: Trans Membrane Pressure

TN: Total Nitrogen

TP: Total Phosphorus

TS: Total Solids

TSS: Total Suspended Solids

UASB: Upflow Anaerobic Sludge Blanket

VFA: Volatile Fatty Acid

VS: Volatile Solids

WWTP: Wastewater Treatment Plant

1. Introduction

Anaerobic treatment in high-rate bioreactors has increased in number of applications during municipal wastewater treatment (MWT) in the last decade while presenting an advanced technology for environmental protection and resource preservation [1,2]. The combination of membrane and an anaerobic bioreactor (Anaerobic membrane bioreactor (AnMBR)) paved the way for a sustainable wastewater treatment with complete biomass retention, and with additional advantages such as less sludge production, high quality effluent and net energy production as the organic matter is converted into high-value products (volatile fatty acids (VFAs)) and energy in the form of biogas [3,4]. These advantages of anaerobic treatment systems result in a decrease in operational costs compared to conventional wastewater treatment plants (WWTPs) that often include aerobic processes (i.e. conventional activated sludge (CAS) or aerobic membrane bioreactor (AeMBR)) [5–7].

Sludge bed-based technologies, such as upflow anaerobic sludge blanket (UASB) reactors, have been widely used for MWT in full-scale WWTPs especially in tropical regions [8–10]. The AnMBRs, on the other hand, have been mainly applied for the treatment of high strength industrial wastewater [1,11]. Currently, emphasis has been given to adapt AnMBRs in MWT to simultaneously recover energy and clean water [12,13]. In fact, AnMBRs are suitable for the treatment of low-loaded wastewater due to the complete retention of slow-growing methanogens and thus have the potential to operate at higher organic loading rates (OLR) [14,15]. Applied OLRs in AnMBRs range from 0.3 $kgCOD \cdot m^{3-1}d^{-1}$ to 12.5 $kgCOD \cdot m^{3-1}d^{-1}$ during the treatment of municipal wastewater [4,16]. From this point of view, further research is still required to optimize the application of AnMBRs in MWT with respect to different feeding characteristics that can help to assess the overall performance of AnMBRs at varying OLRs.

The application of AnMBRs is coastal regions is also another point that lacks sufficient information in the literature for a successful WWTP operation. In coastal regions, variable salinity of wastewater occurs due to seawater infiltration to sewers or introduction of saline water from industrial processes such as seafood and cheese production [12,17]. In general, the salinity effect in anaerobic processes can cause two main critical operational problems. Firstly, increased salinity results in deterioration of membrane filtration and fouling aspects due also to the decrease of the biomass particle size. For instance, salinity increase from 8 20 $gNa^{+}.L^{-}$ to 20 $gNa^{+}.L^{-1}$ was accompanied by the increase of the transmembrane pressure (TMP) up to 350 mbar, while a ten-fold reduction in biomass particle size resulted in a filtration resistance increase [18]. Similarly, Yurtsever et al. [19] indicated that salinity induced large molecules, to be detected as foulants in gel/cake layer; they may originate from biomass loosely bound extracellular polymeric substances (EPS). Secondly, saline conditions can suppress microbial growth and cause the disintegration of flocs and granules that further lead prominent biomass wash-out affecting the sludge granulation [20].

The advantages of AnMBRs in MWT are evident with respect to the necessity of low-cost energy technologies in WWTPs; however, long-term operational experiences in coastal regions are not fully recognized. The main motivation of this study was therefore to investigate the treatment efficiency of low-loaded wastewater in combined sewers by AnMBR and to determine the optimal operating conditions with respect to varying OLRs by using fermented cellulosic sludge rich in VFAs. In this aspect, a pilot-scale UASB coupled with anaerobic ultrafiltration membranes was operated with real influent in a coastal area that is considered hotspot for seawater intrusion, Falconara Marittima WWTP, Italy. The effect of seawater intrusion in biological activity and membrane filtration of municipal wastewater in coastal areas was further elaborated in the specifically-designed integrated treatment scheme. Since the majority of first generation MBRs was implemented in northern Europe, the presented results may offer options to a critical part of the MBR market.

2. Materials and methods

2.1. Full scale plant in Falconara area

The pilot plant is located in the WWTP of Falconara Marittima (coastal area hotspot for seawater intrusion) (**Figure 1**) and fed with the real influent of the full-scale plant. The WWTP of Falconara Marittima (Italy) has a design treatment capacity of 80,000 PE with nominal influent flow rate of 30,000 $m^3 \cdot d^{-1}$. After screening, degritting and primary settling, the wastewater is biologically treated with CAS that is operated with two identical parallel lines applying the Modified Ludzack Ettinger scheme. The total volume of the biological compartment is 13,700 m^3, divided into 8,860 m^3 and 4,900 m^3 for denitrification and nitrification compartments, respectively. The aerated compartments are equipped by ceramic fine bubble diffusers. The Falconara Marittima WWTP is continuously monitored by online sensors (Dissolved Oxygen (DO); temperature; mixed liquor suspended solids (MLSS) and oxidation reduction potential (ORP)) and magnetic flow meters (influent, effluent, recirculation and waste sludge). The average sludge retention time (SRT) is 10 days and the sludge recycle ratio ($Q_{sludge\ recycled}/Q_{influent}$) is 0.5.

The Falconara Marittima WWTP is controlled by the European Environment Agency (www.eea.europa.eu, 2017), since it is located in a coastal area. Since it is a hotspot for infiltrations from groundwater and marine intrusions, low-loading wastewater occurs in the WWTP influent during the dry weather conditions. The average quality of the raw influent is given in **Table 1**; where the values reveal that the influent is characterized by high chloride concentration around 334 ± 236 $mg \cdot L^{-1}$ as a result of the seawater intrusion.

2.2. Pilot plant in Falconara Marittima

Following the preliminary treatment (screening, degritting and oils removal), pretreated influent from Falconara WWTP was sent to a pilot-scale UASB coupled with an anaerobic ultrafiltration membrane. To evaluate the long-term stability period, the experimental work was conducted for 480 days with real wastewater influent. As shown in **Figure 2**, different phases were designed by increasing the influent organic loading testing the following configurations: a) urban wastewater, b) co-treatment of urban wastewater and methanol, c) co-treatment of urban wastewater and fermentation liquid.

A steady influent flow rate of about 3 $L \cdot h^{-1}$ of wastewater and the external addition of methanol and fermented sludge were achieved by peristaltic pumps (Watson-Marlow, UK). The first one was able to guarantee a flow rate of 15 $L \cdot h^{-1}$, the second and the third was 3 $L \cdot h^{-1}$-10 $L \cdot h^{-1}$ and 15 $L \cdot h^{-1}$, respectively.

The UASB was a cylindrical Plexiglas reactor (16 L) with an internal diameter of 15 cm and a total height of 136 cm. The reactor was divided into two compartments: the first was the real reaction chamber at the bottom (85 cm, 12.4 L), while the second, on the top, was a tri-phase separator (GLS) with 21.9 cm height and was connected to a hydraulic guard which created the appropriate backpressure for the biogas release. The temperature of the UASB reactor was kept constant (30˚C) by applying internal and external windings with hot water at 45˚C. The produced biogas was measured by a milligas counter (Ritter, Germany). The hydraulic retention time (HRT) was maintained at 5-6 h. The up-flow velocity of the UASB reactor was maintained at 1 $m \cdot h^{-1}$. The UASB reactor was inoculated with the sludge obtained from a paper mill WWTP in Castelfranco Veneto (Italy).

The UASB effluent was collected in a mixed reactor equipped with pH and temperature probes and it was partially recirculated in the UASB reactor (internal recirculation) and partially sent by gravity to the second unit (anaerobic ultrafiltration tank). The performance of anaerobic process was monitored throughout the experimental period with respect to OLR, indicator α, specific methanogenic activity (SMA), upflow speed and biogas production. The UASB was followed by anaerobic hollow-fiber ultrafiltration membrane (PURON® Koch membrane system, with 0.03 µm of nominal pore-size, a total nominal surface 0.5 m^2, 0.25 m height) installed in a Plexiglas reactor (0.29 m x 0.7 m x 0.39 m) and equipped with level and TMP sensors.

2.3. Experimental periods for UASB and AnMBR

Different OLRs (ranging from 1 $kgCOD\cdot m^{3-1}d^{-1}$ to 2 $kgCOD\cdot m^{3-1}d^{-1}$) were studied and controlled based on the influent flow. All experimental periods, applied configurations and operational parameters are summarized in **Table 2**. In Period 1 only raw wastewater was treated by UASB at 30 ^oC, while the co-treatment of raw wastewater and methanol was tested in Period 2. Period 2 was divided in 3 sub-periods: In Period 2.1, mixture of raw wastewater and methanol was treated only by UASB at 30 ^oC. In Period 2.2, the co-treatment of raw wastewater and methanol was conducted at 30°C UASB + ambient AnMBR; whereas, the same configuration of Period 2.2 was applied in Period 2.3 except that the temperature of AnMBR was also kept at 30 ^oC. In Period 3, the wastewater was co-treated with the supernatant of fermented cellulosic sludge in UASB + AnMBR. During Period 3, the raw wastewater was filtered by dynamic rotating primary unit (SALSNES FS1000) to recover cellulosic sludge [21]. The separated sludge was then sent to anaerobic fermentation reactor (1400 L) operating at 30°C in uncontrolled pH. The fermented flow was dewatered (BABY 2 PIERALISI) and the liquid supernatant was used to increase the OLR of UASB reactor up to 2 $kgCOD\cdot m^{3-1}d^{-1}$. Finally, during the fermentation liquid co-treatment, NaCl solution was added in the UASB reactor to simulate high saline conditions in Period 4. Chloride concentration was increased gradually from 200 $mg\cdot L^{-1}$ to 500 $mg\cdot L^{-1}$ during first 50 days and then to 1500 $mg\cdot L^{-1}$ after 50 days and the maximum of 2200 $mg\cdot L^{-1}$ at the end. The main characteristics of the influents are reported in **Table 3**.

The performance of the system was investigated in terms of organic content removal and biogas production with respect to above-listed configurations (Periods 1-2-3-4). The indicator α, that is defined as the ratio of partial alkalinity over total alkalinity, was measured to verify the stability of the biological process. An upflow velocity of $0.7\ m{\cdot}h^{-1}$ to $1\ m{\cdot}h^{-1}$ was maintained to keep the sludge blanket in suspension.

2.4. Functional characterization of AnMBR

Preliminary tests were carried out to optimize the AnMBR membrane pilot-scale before being coupled with UASB. The start-up conditions of the AnMBR are given in **E-Supplementary material**. The critical flux of the AnMBR was studied through step-flux method and 6 steps of 10 minutes have been tested for 60 minutes. The pump was set up in order to increase the flux rate and TMP was recorded. The critical flux was 12-14 $L{\cdot}m^{2-1}h^{-1}$ and for all the experimental periods membrane flux was maintained below this value. Moreover, the effect of the solid concentration range (0-10 $mgMLSS{\cdot}L^{-1}$, 30-50 $mgMLSS{\cdot}L^{-1}$, 80-100 $mgMLSS{\cdot}L^{-1}$ and 300 $mgMLSS{\cdot}L^{-1}$) in the membrane fouling was assessed. The effect of gas sparging in the fouling rate was evaluated at different solid concentrations with and without gas-sparging to investigate the effect of gas bubbles in terms of membrane fouling and operative TMP values**.** The gas-sparging method adopted in these tests, using nitrogen gas (N_2) for 10 seconds off (gas off) and 10 seconds on (gas on), with a specific flow rate value of 2 $m^3{\cdot}m^{2-1}h^{-1}$ [22]. Moreover, gas-sparging frequency was studied. The results showed that the increase of the gas sparging frequency increased the percentage of degassing methane; and the degassing methane from gas-sparging could be recovered **(**see **E-Supplementary material)**.

For each experimental set, the temperature was measured at the beginning and at the end of the test. The temperature was normalized at 20°C using the Arrhenius equation. For each flux (J) ranging from 6 LMH to 22 LMH, the average TMP (TMP_{ave}) and the slope (dTMP/dt) were calculated **(E-Supplementary material)**. The TMP reached up to 0.79 $mbar{\cdot}min^{-1}$ at MLSS concentration of 300 mgMLSS·L^{-1} without gas sparging. Differently, for the same MLSS concentration, the TMP was maintained below 0.1 mbar·min^{-1} by switching on the gas sparging. Therefore, the gas sparging decreasing the fouling rate independently from the MLSS concentrations and the preliminary tested gas sparging durations were adopted for the long operating periods.

Granulometric characterizations of the influent of AnMBR was conducted to characterize the particle size of influent solids. Accumulative volume percent was calculated. Diameters d_{50} and d_{90} of particles were found to be 14 µm and 58 µm, respectively.

Membrane cleaning was performed by adding hypochlorite solution at 14% w/v (200 ppm) every 45 days to remove organic fouling of the membrane; while NaOCl at a concentration of 1000 mg·L^{-1} or citric acid ($C_6H_8O_7$) at 1000 mg·L^{-1} was used to restore the initial permeability of the membrane. Permeability tests were carried out with tap water and TMP values were measured at different steps of permeation flow rate. Following the preliminary tests, the AnMBR reactor was fed with UASB effluent in different Periods (**Table 2)** and coupled with UASB as mentioned earlier.

2.5. Analytical methods

Standard analyses were conducted in the influent flow, the UASB effluent and the membrane permeate twice a week. All the samples were analyzed in terms of pH, chemical oxygen demand (COD), total Kejdahl nitrogen (TKN), ammonia nitrogen (NH_4^+-N), soluble COD (sCOD), nitrate nitrogen (NO_3-N) and nitrite nitrogen (NO_2-N) according to Standard Methods [23]. The sCOD was measured in the filtrate obtained after the filtration of the sample through 0.45 µm Whatman membrane filters. NO_2-N, NO_3-N were measured by ion chromatography (Dionex DX120) in samples that were first filtered through 0.45 µm Whatman membrane filters.

Moreover, in each period, anaerobic biomass was sampled from UASB reactor to investigate SMA (data not shown) at different OLRs according to the experimental method reported by Hussain et al. [24]. Acetate (solution of 2 $gCOD{\cdot}L^{-1}$, with a ratio VS/COD of 2) was used and its degree of conversion into methane was normalized considering the volatile solids (VS) and expressed in $m^3CH_4{\cdot}kgVS^{-1}d^{-1}$. The CH_4 content of the biogas was analyzed by a Brüel and Kjaer Multi-gas Monitor Type 1302, based on photoacoustic spectroscopy. During the periods with methanol and fermentation liquid addition, extracellular polymeric substances (EPS) were also analyzed according to the method found in Zhang et al. [25].

2.6. Statistical analysis

Principal component analysis (PCA) was applied to the dataset to identify the relationships between the applied operating conditions and system performance. The obtained loadings of the variables in each principal component (**see E-Supplementary material**) mapped their relationship with the respective principal component (PC). The scores of the principal components mapped the different samples in the new dimensional space of the principal components that simplified the investigation of the different relationships between the variables. The first two principal components (PC1 and PC2) were then selected for further interpretation of the results. More information on the PCA can be found in [26].

3. Results and discussion

3.1. Characterization and variability of the influent

The main characteristics of the influent for each operational period is given in **Table 3**. pH of the influent remained almost stable (7.5 - 7.8). The total alkalinity of the influent ranged from 281 ± 92 $mgCaCO_3{\cdot}L^{-1}$ to 526 ± 110 $mgCaCO_3{\cdot}L^{-1}$, with the highest concentration observed in Period 3. EC was 1464 ± 153 ms/cm and 1467 ± 380 ms/cm in Period 1 and Period 2 respectively, while the addition the fermentation liquid increased the EC to 1817 ± 350 $ms{\cdot}cm^{-1}$ in Period 3. Saline conditions in Period 4 increased further the EC up to 2787 ± 1300 $ms{\cdot}cm^{-}$

[1]. Cl^- concentration of the influent was 282 ± 126 mg·L^{-1}, 304 ± 300 mg·L^{-1}, 393 ± 377 mg·L^{-1} and 1100 ± 618 mg·L^{-1} during Periods 1, 2, 3 and 4, respectively. COD concentrations were 207 ± 73 mg·L^{-1}, 388 ± 80 mg·L^{-1}, 375 ± 148 mg·L^{-1} and 550±330 mg·L^{-1} in Periods 1,2,3 and 4, respectively. Meanwhile, the average sCOD concentration was 50 mg·L^{-1} in Period 1 and in the range of 242-297 mg·L^{-1} in Periods 2, 3 and 4. The addition of methanol and fermentation liquid increased the soluble organic fraction of the influent. On the other hand, the soluble organic fraction in the influent decreased due to the saline conditions occurred in Period 4. NH_4^+-N concentration was 22±6 mg·L^{-1}, 20.5±6 mg·L^{-1}, 61±33 mg·L^{-1} and 30±10 mg·L^{-1} in Periods 1, 2, 3 and 4, respectively. The highest influent TP and PO_4-P concentrations were observed in Period 3, while the highest TSS concentration was recorded in Period 4. The results are in line with existing literature that were reported for typical real municipal wastewater, except for parameters such as EC, TSS, Cl^- and SO_4^{2-} that were measured comparatively higher due to seawater intrusion in the WWTP of Falconara Marittima [3,5,27].

3.2. Start-up period of UASB

The UASB reactor was inoculated with anaerobic sludge (granular sludge TS = 2.72%, VS/TS = 74%; flocculent sludge TS = 1.42%, VS/TS = 60%) taken from a paper mill WWTP in Castelfranco Veneto, Italy. The temperature of the UASB was increased gradually to 30°C after 10 days of operation period. The reactor operated for 5 months at 30°C with OLR value of 1.05 ± 0.4 kgCOD·m$^{3-1}$d^{-1}. The flow rate was maintained between at 3.38 ± 0.6 L·h^{-1}. Meanwhile, α was between 0.12 and 0.43, which indicated a stable biological process. The average biogas production was 0.39 ± 0.2 Lbiogas·d^{-1}. The COD and TSS removal efficiencies were 63% and 84%, respectively. In addition, 86% of P and N were released during the start-up period of UASB.

3.3. Effect of OLR in the system performance

Following the start-up period, the UASB was first fed with the mixture of municipal wastewater and methanol (as the external C source) and the OLR was increased to 2.1 ± 0.6 $kgCOD{\cdot}m^{3-1}d^{-1}$ (Period 2). The flow rate was maintained at 2.98 ± 0.3 $L{\cdot}h^{-1}$. The variations in OLR and α value throughout the operation period are given in **Figure 3a** and **Figure 3b**, respectively. α value remained almost stable in the beginning of Period 2, and then tended to increase up to 0.65. while 86% P and 88% N were released. The average biogas production increased to 2.8 ± 1.5 $Lbiogas{\cdot}d^{-1}$ with 33.6% ± 10.5% of CH_4 in Period 2. The COD and TSS removal efficiencies of the UASB was 70% and 48% respectively while the application of the AnMBR increased the average COD and TSS removal to 85%, and > 99.99%. On the other hand, the release of P and N in the UASB was 86% P and 88% N and then slightly decreased to 76% and 83% in the integrated UASB+AnMBR configuration, respectively.

In Period 3, α value was between 0.16 and 0.52 with an average of 0.29 ± 0.1. The addition of the fermentation liquid in the influent resulted in a peak in the biogas production; while only 9% CH_4 was measured indicating excess CO_2 production via fermentation (**Figure 4**). The biogas production started to increase gradually together with the CH_4 content of the biogas. Hence, up to 10.25 $Lbiogas{\cdot}d^{-1}$ was generated with 51.9% of CH_4 (average of 4.11 ± 3.1 $Lbiogas{\cdot}d^{-1}$ with 29.7% ± 11.8% of CH_4). The addition of fermentation liquid as the external carbon source increased the biogas production without affecting the overall CH_4 content of the biogas. In Period 3, the COD removal efficiencies were 42% and 83% in UASB and UASB+AnMBR, respectively. The TSS removal efficiencies in UASB and UASB+AnMBR were 38% and 100%, respectively, while P and N releases were 85% and 75%, respectively.

The application of the AnMBR in the study of Gouveia et al. [2] for the treatment of municipal wastewater under psychrophilic conditions and loading rate of 2 and 2.5 $kgtCOD{\cdot}m^{3-1}d^{-1}$ resulted in effluent tCOD concentrations of 100 $mg{\cdot}L^{-1}$–120 $mg{\cdot}L^{-1}$. In another study by Wei et al. [28], a wide range of volumetric OLR (0.8–10 $gCOD{\cdot}L^{-1}d^{-1}$) was tested in AnMBR to treat synthetic municipal wastewater. The results showed that at steady conditions, 98% COD removal was achieved while the application of high sludge OLR led to high methane production of over 300 $mL\cdot gCOD^{-1}$. Wijekoon et al. [29] tested the performance of a thermophilic AnMBR at different OLRs ranging from 5 12 $kgCOD\cdot m^{3-1}d^{-1}$ to 12 $kgCOD\cdot m^{3-1}d^{-1}$. The authors reported an average biogas production of 15 $L\cdot d^{-1}$, 20 $L\cdot d^{-1}$ and 35 $L\cdot d^{-1}$ at OLRs of 5.1 ± 0.1 $kgCOD\cdot m^{3-1}d^{-1}$, 8.1 ± 0.3 $kgCOD\cdot m^{3-1}d^{-1}$ and 12.0 ± 0.2 $kgCOD\cdot m^{3-1}d^{-1}$, respectively, with CH_4 content of about 55%–65%. In addition, the reactor showed optimum COD removal efficiencies at 8 ± 0.3 $kgCOD\cdot m^{3-1}d^{-1}$ OLR. In a recent study, the highest VFA yield (48.20 ± 1.21 $mgVFA\cdot 10$ $mgCOD_{feed}$) was observed at OLR of 550 $mgCOD\cdot L^{-1}$; however, the authors achieved less VFA yield at the examined maximum OLR (715 $mgCOD\cdot L^{-1}$), indicating that elevated OLRs can lead to high VFA production but it is also crucial to optimize operating OLR during the treatment of low strength wastewater in AnMBR [30]. In high-rate bioreactors such as UASB and AnMBR, VFAs may not be efficiently converted to methane due to low-retention times and can accumulate in the reactor and thus can be detected in the effluent [1,31]. In operating conditions at elevated OLRs, VFAs should be therefore monitored to meet the local standards for discharge or reuse.

3.4. Effect of salinity in the system performance

The impact of high salinity conditions was assessed in Period 4. Chloride concentration during all operational periods are given in **Figure 5**. The red points mark the membrane cleaning days. α value was stable around 0.3. Biogas production gradually decreased with increasing Cl^- concentrations (see **Figure 4)**. Although the biogas production was 1.2-1.3 $L\cdot d^{-1}$ during in the beginning of Period 4 (so-called initial low saline conditions at 200 $mgCl^-\cdot L^{-1}$), it decreased to 0.13-0.57 $L\cdot d^{-1}$ in the presence of 500 $mgCl^-\cdot L^{-1}$. When the Cl^- concentration was increased to 1500 $mg\cdot L^{-1}$ (at the end of Period 4), the CH_4 content in biogas reduced by 27% compared to the reported values under low saline conditions. The CH_4 content of the biogas was adversely affected (10%-20% in the beginning of Period 4 and 5% at the end) by high saline conditions. The system almost failed to operate at the maximum examined Cl^- concentration (app. 2200 $mg \cdot L^{-1}$), since the biogas production was 0.08 $L \cdot d^{-1}$ with 3% CH_4.

In line with the results of the current study, Aslan et al. [32] demonstrated that the COD removal significantly decreased at about 20 $gNa^+ \cdot L^{-1}$ when treating saline wastewater in a UASB reactor. Reduced biogas production and COD removal were also reported by Song et al. [12] in an AnMBR operating under saline conditions 15 $gNa^+ \cdot L^{-1}$. A decrease in biomass production was observed with the increase of salinity in the AnMBR. A NaCl shock load (increase from 5 $gNaCl \cdot L\text{-}^1$ to 60 $gNaCl \cdot L\text{-}^1$) caused a reduction of COD and TKN removal efficiencies in the study of Yogalakshmi et al. [17]; COD and TKN removal dropped to 64% and 23% at 60 $gNaCl \cdot L\text{-}^1$, respectively, while the nitrification was completely inhibited. In the study of Luo et al [33], a reduction of TOC and NH_4^+-N removals was initially observed with elevated NaCl loading; however, microbial diversity in saline AnMBR did not change and the adaptation of microbial community to saline conditions was stated to recover AnMBR biological performance. In a recent work of Muñoz Sierra et al. [34], the performance of UASB and AnMBR was evaluated for the treatment of highly-saline phenolic wastewater. The authors highlighted the superiority of AnMBR over UASB in terms of bioreactor conversion, biomass characteristics and microbial community under salinity up to 26 $gNa^+ \cdot L^{-1}$ due to its greater probability to maintain functionality and to respond to high salinity. In another study of the same authors [18], a short-term salinity fluctuation of 18 $gNa^+ \cdot L^{-1}$ to 20 $gNa^+ \cdot L^{-1}$ did not affect the long term operation of AnMBR. The results indicated that high saline conditions initially caused a decrease of the biological performance of AnMBR; however, long term adaptation of microbial community to saline conditions (i.e. halotolerant or even halophilic microorganisms) is required with high biomass concentrations for the system to regain its stability.

3.5. Effect of OLR and high salinity on membrane operation

The variations in the TMP value is shown in **Figure 6a**. The red points mark the membrane cleaning days. The TMP of the membrane was stable at around 50 mbar when the system was operated with methanol addition (Period 2, OLR of 2 $kgCOD{\cdot}m^{3-1}d^{-1}$), with gas-sparging condition of 10 seconds on and 120 seconds off. The specific flux normalized at 20°C was 175 $L{\cdot}h^{-1}m^{2-1}bar^{-1}$ and only NaOCl cleaning was necessary after 50 days of operation. (**Figure 6b**) During the fermentation liquid addition (Period 3, OLR of 2 $kgCOD{\cdot}m^{3-1}d^{-1}$) the behavior was first similar to the methanol co-treatment; then the TMP increased gradually after 50 days of operation and reached to 500 mbar after 100 days of operation. Thus, a more intense chemical cleaning was applied to restore the initial permeability (citric acid at 1000 $mg{\cdot}L^{-1}$) on day 315. A significant EPS production was observed when the fermentation liquid was used, leading to larger formation of the "cake" on the surface of the membrane. This was mainly due to the fluctuation of the characteristics of the production of fermentation liquid. There was an increase in EPS concentration from 52.8 $mgEPS{\cdot}L^{-1}$ in the Period 2 to 70.8 $mgEPS{\cdot}L^{-1}$ in the Period 3. The latter decreased the membrane permeability a short time, higher rate pore obstruction and therefore more intense and frequent cleaning was required.

In Period 4, the TMP remained stable at 12 mbar when the reactors were fed with the fermentation liquid together with additional NaCl to increase salinity in the system, at the concentration of 500 $mgCl^{-}{\cdot}L^{-1}$. The average TMP was around 50 mbar, at Cl^{-} concentration of 1500 $mgCl^{-}{\cdot}L^{-1}$, caused by the increased filtration resistance following the increased TMP due to the increased EPS concentration in Period 3. The saline conditions therefore only affected the initial TMP value and remained constant during the system operation.

The TMP of AnMBRs is highly dependent on the critical flux and the sparging rate together with other environmental and operating conditions [35]. Furthermore, an initial flux below the critical flux, prior to the introduction of peak flow, is reported to be advantageous to permeability recovery [36]. In the study of Muñoz Sierra et al. [18], increased salt concentration was found to affect the TMP negatively (350 mbar at a flux of 4.0 $Lm^{2}\cdot h^{-1}$). The deterioration of membrane filtration performance was attributed to the decrease of biomass particle size when salinity was increased. The small particle size had a significant influence on the cake layer compaction that increased the operational values of the filtration resistance. Furthermore, higher stability of process performances of AnMBR over UASB was reported to overcome high salinity [34]. Elevated TMP values were also reported by Yurtsever et al. [19] with respect to high salinity conditions. The salinity induced large molecules as foulants in gel/cake layer, that may originate from biomass loosely bound EPS. The EPS properties are highly dependent on the operating OLR [37]; and the OLR increase is often accompanied with high EPS production.

3.6. Relationship between process parameters and system performance

PCA was carried out to reveal the relationships between the applied operating conditions (i.e. OLR, salinity) and system performance in terms of biogas production, CH_4 content of the biogas, α value, TMP and Js. The PCA supported our previous discussion regarding the performance of the UASB+AnMBR system at different periods. OLR was closely grouped together with the biogas production, CH_4 content and α value (**Figure 7**). This cluster showed the close relationship between these parameters mostly in Period 1 which included only the UASB operation. The data-points of Period 2 was comparatively more equally distributed between the parameters; where the negative impacts of OLR on TMP were clearly reflected in our data especially in Period 3. Furthermore, data points of Period 4 were characterized by high chloride concentrations. The displayed negative correlation between the salinity and TMP and/or Js in Period 4 was due the citric acid cleaning of the membrane following the fouling occurred in Period 3 that was previously mentioned. TMP value was close to its initial value right after the citric acid cleaning and although TMP also slightly increased Period 4 at high chloride concentrations (**see E-Supplementary material**), this increase was relatively low comparing to Period 3.

3.7. Assessment of the possible re-use of the effluent

Population density and economic activity lead to significant differences in the water stress levels of the basins. Water stress occurs in many areas of the EU, particularly in the Mediterranean regions and part of the Atlantic regions. The data indicated by the European Environment Agency regarding the use of water in various sectors indicate that irrigation in agriculture represents about half of the water used annually with high seasonal and geographical variations.

In May 2018, the European Commission presented a proposal for the reuse of treated wastewater through a regulation that establishes common minimum requirements. This proposal was going under revision with resolution of 12th February 2019. The proposal imposes obligations that include:

• Compliance with the minimum requirements. These requirements differ based on four water quality classes defined according to the type of crop and the irrigation method.

• Monitoring of recovered water based on minimum test frequency requirements.

The resolution also provides that the competent authorities of the member states can impose additional requirements based on a risk management plan presented by the water utility to mitigate unacceptable risks to health and environment.

In Italy, at national level, D.M. 185 of 2003 establishes the minimum quality requirements for the reclaimed wastewater and its reuse, including limits on nitrogen and phosphorus.

The obtained effluent quality was compared against the values reported in **Table 4** (minimum requirements for reuse). Analyzing the results obtained from this study, the UASB effluent is not compliant with the limits required both by D.M. 185/03 and by the new EU proposal. The permeate of the AnMBR appears of higher quality because it is free from TSS and falls into class A since ultrafiltration guarantees also disinfection and *E. coli* removal. For the restrictions posed by the D.M. 185/2003 for the reuse of the treated wastewater are mainly related to the high concentration of chloride (High values are due to the salt water intrusion since the plant is in a coastal area) and the nutrients concentration (N and P), both exceed the limit values. The high nutrient content in the permeate (TN reaches a maximum value of 60 $mgN \cdot L^{-1}$, TP of 6 $mgP \cdot L^{-1}$), allows a potential reuse in fertigation field.

The national law in Italy is more restrictive compared to the new European proposal, which introduces an innovative approach based on a framework for the risk management. EU requirements vary according to the type of irrigated crops and according to the method of irrigation, taking into account the potential risk of contamination of the products. The most strict requirements are provided for class A, where reclaimed water can be in contact with the edible parts of irrigated food crops.

4. Conclusions

Regarding the change of paradigm in the context of circular economy in terms of water, nutrients and energy; site-specific optimization of an AnMBR is crucial especially in coastal regions. In this particular study, the addition of fermented cellulosic sludge to raw wastewater increased the biogas production without affecting the overall CH_4 content of the biogas. In case of the application of the process in coastal areas, the biogas production decreased by 27% due to the saline conditions when Cl concentration was up to 1500 $mg \cdot L^{-1}$. Moreover, the CH_4 content of the biogas was also adversely related to the high saline conditions up to almost null value for chloride higher than 2000 $mg \cdot L^{-1}$. At Cl^- concentration less than 1500 $mgCl \cdot L^{-1}$, long term adaptation of microbial community (i.e. halotolerant or even halophilic microorganisms) may be required with high biomass concentrations for the system to regain its stability and recover the bioreactor performance in UASB+SuMBR. Concerning the membrane operation, the increased salinity resulted only in the increase of initial TMP value where it stayed constant during the following operating days. The AnMBR effluent fell into class A since ultrafiltration guarantees also disinfection and *E. coli* removal.

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Appendix A. Supplementary data

Supplementary data to this article can be found in the online version.

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Figure Captions

Fig. 1. Salt water intrusion (European Environment Agency, 2017) and the location of Falconara Marittima WWTP.

Fig. 2. Scheme of the UASB pilot plant coupled with anaerobic ultrafiltration membranes: Configuration with a) urban wastewater, b) co-treatment of urban wastewater and methanol, c) co-treatment of urban wastewater and fermentation liquid.

Fig. 3. Variations in a) OLR b) α value (the ratio of partial alkalinity over total alkalinity) at different periods of the operational time.

Fig. 4. Variations in a) Biogas production b) CH_4 content of the biogas at different periods of the operational time.

Fig. 5. Variations in chloride concentration at different periods of the operational time.

Fig. 6. Variations in a) TMP profile b) Specific flux at 20^{o}C in AnMBR at different periods of the operational time. The red points mark the membrane cleaning days. Citric acid cleaning was conducted on day 315. TMP and specific flux were not recorded between the days 315 and 432.

Fig. 7. Principal component analysis (PCA) of the operating parameters and system performance.