

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

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## 1 **Abstract**

2 Long term operation of an anaerobic membrane bioreactor (AnMBR) treating municipal  
3 wastewater was investigated in a real seawater intrusion spot in Falconara Marittima (Central  
4 Italy) on the Adriatic coastline. Changes in biological conversion and system stability were  
5 determined with respect to varying organic loading rate (OLR) and high salinity conditions. At  
6 an OLR of  $1 \text{ kgCOD}\cdot\text{m}^3\cdot\text{d}^{-1}$ , biogas production was around  $0.39 \pm 0.2 \text{ L}\cdot\text{d}^{-1}$ . The increase of  
7 the OLR to  $2 \text{ kgCOD}\cdot\text{m}^3\cdot\text{d}^{-1}$  resulted in the increase of biogas production to  $2.8 \pm 1.5 \text{ L}\cdot\text{d}^{-1}$   
8 (with  $33.6\% \pm 10.5\%$  of  $\text{CH}_4$ ) with methanol addition and to  $4.11 \pm 3.1 \text{ L}\cdot\text{d}^{-1}$  (with  $29.7\% \pm$   
9  $11.8\%$  of  $\text{CH}_4$ ) with fermented cellulosic sludge addition. COD removal by the AnMBR was  
10  $83\% \pm 1\%$  when the effluent COD concentration was below  $100 \text{ mg O}_2\cdot\text{L}^{-1}$ . The addition of the  
11 fermented sludge affected the membrane operation; significant fouling occurred after long-term  
12 filtration, where the trans-membrane pressure (TMP) reached up to 500 mbar. Citric acid  
13 solution was applied to remove scalants and the TMP reached the initial value. High saline  
14 conditions of  $1500 \text{ mgCl}\cdot\text{L}^{-1}$  adversely affected the biogas production without deteriorating the  
15 membrane operation. The treated effluent met the EU quality standards of the D.M. 185/2003  
16 and the new European Commission Resolution for reuse in agriculture.

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

19

## 20 **Nomenclature**

21 AD: Anaerobic Digestion

22 AeMBR: Aerobic Membrane Bioreactor

23 AnMBR: Anaerobic Membrane Bioreactor

24 CAS: Conventional Activated Sludge

25 COD: Chemical Oxygen Demand

- 1 DO: Dissolved Oxygen
- 2 EC: Electrical Conductivity
- 3 EPS: Extracellular Polymeric Substances
- 4 HRT: Hydraulic Retention Time
- 5 MLSS: Mixed Liquor Suspended Solids
- 6 MWT: Municipal Wastewater Treatment
- 7 OLR: Organic Loading Rate
- 8 ORP: Oxidation Reduction Potential
- 9 PE: Population Equivalent
- 10 sCOD: Soluble Chemical Oxygen Demand
- 11 SMA: Specific Methanogenic Activity
- 12 SRT: Sludge Retention Time
- 13 SuMBR: Submerged Membrane Bioreactor
- 14 tCOD: Total Chemical Oxygen Demand
- 15 TKN: Total Kjeldahl Nitrogen
- 16 TMP: Trans Membrane Pressure
- 17 TN: Total Nitrogen
- 18 TP: Total Phosphorus
- 19 TS: Total Solids
- 20 TSS: Total Suspended Solids
- 21 UASB: Upflow Anaerobic Sludge Blanket
- 22 VFA: Volatile Fatty Acid
- 23 VS: Volatile Solids
- 24 WWTP: Wastewater Treatment Plant
- 25

## 1 **1. Introduction**

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

13 Sludge bed-based technologies, such as upflow anaerobic sludge blanket (UASB) reactors, have  
14 been widely used for MWT in full-scale WWTPs especially in tropical regions [8–10]. The  
15 AnMBRs, on the other hand, have been mainly applied for the treatment of high strength  
16 industrial wastewater [1,11]. Currently, emphasis has been given to adapt AnMBRs in MWT  
17 to simultaneously recover energy and clean water [12,13]. In fact, AnMBRs are suitable for the  
18 treatment of low-loaded wastewater due to the complete retention of slow-growing  
19 methanogens and thus have the potential to operate at higher organic loading rates (OLR)  
20 [14,15]. Applied OLRs in AnMBRs range from  $0.3 \text{ kgCOD}\cdot\text{m}^3\cdot\text{d}^{-1}$  to  $12.5 \text{ kgCOD}\cdot\text{m}^3\cdot\text{d}^{-1}$   
21 during the treatment of municipal wastewater [4,16]. From this point of view, further research  
22 is still required to optimize the application of AnMBRs in MWT with respect to different  
23 feeding characteristics that can help to assess the overall performance of AnMBRs at varying  
24 OLRs.

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

15 The advantages of AnMBRs in MWT are evident with respect to the necessity of low-cost  
16 energy technologies in WWTPs; however, long-term operational experiences in coastal regions  
17 are not fully recognized. The main motivation of this study was therefore to investigate the  
18 treatment efficiency of low-loaded wastewater in combined sewers by AnMBR and to  
19 determine the optimal operating conditions with respect to varying OLRs by using fermented  
20 cellulosic sludge rich in VFAs. In this aspect, a pilot-scale UASB coupled with anaerobic  
21 ultrafiltration membranes was operated with real influent in a coastal area that is considered  
22 hotspot for seawater intrusion, Falconara Marittima WWTP, Italy. The effect of seawater  
23 intrusion in biological activity and membrane filtration of municipal wastewater in coastal areas  
24 was further elaborated in the specifically-designed integrated treatment scheme. Since the

1 majority of first generation MBRs was implemented in northern Europe, the presented results  
2 may offer options to a critical part of the MBR market.

## 3 **2. Materials and methods**

### 4 **2.1. Full scale plant in Falconara area**

5 The pilot plant is located in the WWTP of Falconara Marittima (coastal area hotspot for  
6 seawater intrusion) (**Figure 1**) and fed with the real influent of the full-scale plant. The WWTP  
7 of Falconara Marittima (Italy) has a design treatment capacity of 80,000 PE with nominal  
8 influent flow rate of 30,000 m<sup>3</sup>·d<sup>-1</sup>. After screening, degritting and primary settling, the  
9 wastewater is biologically treated with CAS that is operated with two identical parallel lines  
10 applying the Modified Ludzack Ettinger scheme. The total volume of the biological  
11 compartment is 13,700 m<sup>3</sup>, divided into 8,860 m<sup>3</sup> and 4,900 m<sup>3</sup> for denitrification and  
12 nitrification compartments, respectively. The aerated compartments are equipped  
13 by ceramic fine bubble diffusers. The Falconara Marittima WWTP is continuously monitored  
14 by online sensors (Dissolved Oxygen (DO); temperature; mixed liquor suspended solids  
15 (MLSS) and oxidation reduction potential (ORP)) and magnetic flow meters  
16 (influent, effluent, recirculation and waste sludge). The average sludge retention time (SRT) is  
17 10 days and the sludge recycle ratio ( $Q_{\text{sludge recycled}}/Q_{\text{influent}}$ ) is 0.5.

18 The Falconara Marittima WWTP is controlled by the European Environment Agency  
19 ([www.eea.europa.eu](http://www.eea.europa.eu), 2017), since it is located in a coastal area. Since it is a hotspot for  
20 infiltrations from groundwater and marine intrusions, low-loading wastewater occurs in the  
21 WWTP influent during the dry weather conditions. The average quality of the raw influent is  
22 given in **Table 1**; where the values reveal that the influent is characterized by high chloride  
23 concentration around  $334 \pm 236$  mg·L<sup>-1</sup> as a result of the seawater intrusion.

### 24 **2.2. Pilot plant in Falconara Marittima**

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

8 A steady influent flow rate of about  $3 \text{ L}\cdot\text{h}^{-1}$  of wastewater and the external addition of methanol  
9 and fermented sludge were achieved by peristaltic pumps (Watson-Marlow, UK). The first one  
10 was able to guarantee a flow rate of  $15 \text{ L}\cdot\text{h}^{-1}$ , the second and the third was  $3 \text{ L}\cdot\text{h}^{-1}$ - $10 \text{ L}\cdot\text{h}^{-1}$  and  
11  $15 \text{ L}\cdot\text{h}^{-1}$ , respectively.

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

22 The UASB effluent was collected in a mixed reactor equipped with pH and temperature probes  
23 and it was partially recirculated in the UASB reactor (internal recirculation) and partially sent  
24 by gravity to the second unit (anaerobic ultrafiltration tank). The performance of anaerobic  
25 process was monitored throughout the experimental period with respect to OLR, indicator  $\alpha$ ,

1 specific methanogenic activity (SMA), upflow speed and biogas production. The UASB was  
2 followed by anaerobic hollow-fiber ultrafiltration membrane (PURON® Koch membrane  
3 system, with 0.03  $\mu\text{m}$  of nominal pore-size, a total nominal surface 0.5  $\text{m}^2$ , 0.25 m height)  
4 installed in a Plexiglas reactor (0.29 m x 0.7 m x 0.39 m) and equipped with level and TMP  
5 sensors.

### 6 **2.3. Experimental periods for UASB and AnMBR**

7 Different OLRs (ranging from 1  $\text{kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$  to 2  $\text{kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ ) were studied and  
8 controlled based on the influent flow. All experimental periods, applied configurations and  
9 operational parameters are summarized in **Table 2**. In Period 1 only raw wastewater was treated  
10 by UASB at 30 °C, while the co-treatment of raw wastewater and methanol was tested in Period  
11 2. Period 2 was divided in 3 sub-periods: In Period 2.1, mixture of raw wastewater and methanol  
12 was treated only by UASB at 30 °C. In Period 2.2, the co-treatment of raw wastewater and  
13 methanol was conducted at 30°C UASB + ambient AnMBR; whereas, the same configuration  
14 of Period 2.2 was applied in Period 2.3 except that the temperature of AnMBR was also kept at  
15 30 °C. In Period 3, the wastewater was co-treated with the supernatant of fermented cellulosic  
16 sludge in UASB + AnMBR. During Period 3, the raw wastewater was filtered by dynamic  
17 rotating primary unit (SALSNES FS1000) to recover cellulosic sludge [21]. The separated  
18 sludge was then sent to anaerobic fermentation reactor (1400 L) operating at 30°C in  
19 uncontrolled pH. The fermented flow was dewatered (BABY 2 PIERALISI) and the liquid  
20 supernatant was used to increase the OLR of UASB reactor up to 2  $\text{kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ . Finally,  
21 during the fermentation liquid co-treatment, NaCl solution was added in the UASB reactor to  
22 simulate high saline conditions in Period 4. Chloride concentration was increased gradually  
23 from 200  $\text{mg}\cdot\text{L}^{-1}$  to 500  $\text{mg}\cdot\text{L}^{-1}$  during first 50 days and then to 1500  $\text{mg}\cdot\text{L}^{-1}$  after 50 days and  
24 the maximum of 2200  $\text{mg}\cdot\text{L}^{-1}$  at the end. The main characteristics of the influents are reported  
25 in **Table 3**.



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

#### 6 **2.4. Functional characterization of AnMBR**

7 Preliminary tests were carried out to optimize the AnMBR membrane pilot-scale before being  
8 coupled with UASB. The start-up conditions of the AnMBR are given in **E-Supplementary**  
9 **material**. The critical flux of the AnMBR was studied through step-flux method and 6 steps of  
10 10 minutes have been tested for 60 minutes. The pump was set up in order to increase the flux  
11 rate and TMP was recorded. The critical flux was  $12\text{-}14 \text{ L}\cdot\text{m}^2\cdot\text{h}^{-1}$  and for all the experimental  
12 periods membrane flux was maintained below this value. Moreover, the effect of the solid  
13 concentration range ( $0\text{-}10 \text{ mgMLSS}\cdot\text{L}^{-1}$ ,  $30\text{-}50 \text{ mgMLSS}\cdot\text{L}^{-1}$ ,  $80\text{-}100 \text{ mgMLSS}\cdot\text{L}^{-1}$  and  $300$   
14  $\text{mgMLSS}\cdot\text{L}^{-1}$ ) in the membrane fouling was assessed. The effect of gas sparging in the fouling  
15 rate was evaluated at different solid concentrations with and without gas-sparging to investigate  
16 the effect of gas bubbles in terms of membrane fouling and operative TMP values. The gas-  
17 sparging method adopted in these tests, using nitrogen gas ( $\text{N}_2$ ) for 10 seconds off (gas off) and  
18 10 seconds on (gas on), with a specific flow rate value of  $2 \text{ m}^3\cdot\text{m}^2\cdot\text{h}^{-1}$  [22]. Moreover, gas-  
19 sparging frequency was studied. The results showed that the increase of the gas sparging  
20 frequency increased the percentage of degassing methane; and the degassing methane from gas-  
21 sparging could be recovered (see **E-Supplementary material**).

22 For each experimental set, the temperature was measured at the beginning and at the end of the  
23 test. The temperature was normalized at  $20^\circ\text{C}$  using the Arrhenius equation. For each flux (J)  
24 ranging from 6 LMH to 22 LMH, the average TMP ( $\text{TMP}_{\text{ave}}$ ) and the slope ( $d\text{TMP}/dt$ ) were  
25 calculated (**E-Supplementary material**). The TMP reached up to  $0.79 \text{ mbar}\cdot\text{min}^{-1}$  at MLSS

1 concentration of  $300 \text{ mgMLSS}\cdot\text{L}^{-1}$  without gas sparging. Differently, for the same MLSS  
2 concentration, the TMP was maintained below  $0.1 \text{ mbar}\cdot\text{min}^{-1}$  by switching on the gas sparging.  
3 Therefore, the gas sparging decreasing the fouling rate independently from the MLSS  
4 concentrations and the preliminary tested gas sparging durations were adopted for the long  
5 operating periods.

6 Granulometric characterizations of the influent of AnMBR was conducted to characterize the  
7 particle size of influent solids. Accumulative volume percent was calculated. Diameters  $d_{50}$  and  
8  $d_{90}$  of particles were found to be  $14 \mu\text{m}$  and  $58 \mu\text{m}$ , respectively.

9 Membrane cleaning was performed by adding hypochlorite solution at 14% w/v (200 ppm)  
10 every 45 days to remove organic fouling of the membrane; while NaOCl at a concentration of  
11  $1000 \text{ mg}\cdot\text{L}^{-1}$  or citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ) at  $1000 \text{ mg}\cdot\text{L}^{-1}$  was used to restore the initial permeability  
12 of the membrane. Permeability tests were carried out with tap water and TMP values were  
13 measured at different steps of permeation flow rate. Following the preliminary tests, the  
14 AnMBR reactor was fed with UASB effluent in different Periods (**Table 2**) and coupled with  
15 UASB as mentioned earlier.

## 16 **2.5. Analytical methods**

17 Standard analyses were conducted in the influent flow, the UASB effluent and the membrane  
18 permeate twice a week. All the samples were analyzed in terms of pH, chemical oxygen  
19 demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ), soluble COD  
20 (sCOD), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and nitrite nitrogen ( $\text{NO}_2\text{-N}$ ) according to Standard Methods  
21 [23]. The sCOD was measured in the filtrate obtained after the filtration of the sample through  
22  $0.45 \mu\text{m}$  Whatman membrane filters.  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$  were measured by ion chromatography  
23 (Dionex DX120) in samples that were first filtered through  $0.45 \mu\text{m}$  Whatman membrane  
24 filters.

1 Moreover, in each period, anaerobic biomass was sampled from UASB reactor to investigate  
2 SMA (data not shown) at different OLRs according to the experimental method reported by  
3 Hussain et al. [24]. Acetate (solution of  $2 \text{ gCOD}\cdot\text{L}^{-1}$ , with a ratio VS/COD of 2) was used and  
4 its degree of conversion into methane was normalized considering the volatile solids (VS) and  
5 expressed in  $\text{m}^3\text{CH}_4\text{kgVS}^{-1}\text{d}^{-1}$ . The  $\text{CH}_4$  content of the biogas was analyzed by a Brüel and  
6 Kjaer Multi-gas Monitor Type 1302, based on photoacoustic spectroscopy. During the periods  
7 with methanol and fermentation liquid addition, extracellular polymeric substances (EPS)  
8 were also analyzed according to the method found in Zhang et al. [25].

## 9 **2.6. Statistical analysis**

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

## 18 **3. Results and discussion**

### 19 **3.1. Characterization and variability of the influent**

20 The main characteristics of the influent for each operational period is given in **Table 3**. pH of  
21 the influent remained almost stable (7.5 - 7.8). The total alkalinity of the influent ranged from  
22  $281 \pm 92 \text{ mgCaCO}_3\text{L}^{-1}$  to  $526 \pm 110 \text{ mgCaCO}_3\text{L}^{-1}$ , with the highest concentration observed in  
23 Period 3. EC was  $1464 \pm 153 \text{ ms/cm}$  and  $1467 \pm 380 \text{ ms/cm}$  in Period 1 and Period 2  
24 respectively, while the addition the fermentation liquid increased the EC to  $1817 \pm 350 \text{ ms}\cdot\text{cm}^{-1}$   
25 <sup>1</sup> in Period 3. Saline conditions in Period 4 increased further the EC up to  $2787 \pm 1300 \text{ ms}\cdot\text{cm}^{-1}$

1 <sup>1</sup>. Cl<sup>-</sup> concentration of the influent was 282 ± 126 mg·L<sup>-1</sup>, 304 ± 300 mg·L<sup>-1</sup>, 393 ± 377 mg·L<sup>-1</sup>  
2 and 1100 ± 618 mg·L<sup>-1</sup> during Periods 1, 2, 3 and 4, respectively. COD concentrations were 207  
3 ± 73 mg·L<sup>-1</sup>, 388 ± 80 mg·L<sup>-1</sup>, 375 ± 148 mg·L<sup>-1</sup> and 550±330 mg·L<sup>-1</sup> in Periods 1,2,3 and 4,  
4 respectively. Meanwhile, the average sCOD concentration was 50 mg·L<sup>-1</sup> in Period 1 and in the  
5 range of 242-297 mg·L<sup>-1</sup> in Periods 2, 3 and 4. The addition of methanol and fermentation liquid  
6 increased the soluble organic fraction of the influent. On the other hand, the soluble organic  
7 fraction in the influent decreased due to the saline conditions occurred in Period 4. NH<sub>4</sub><sup>+</sup>-N  
8 concentration was 22±6 mg·L<sup>-1</sup>, 20.5±6 mg·L<sup>-1</sup>, 61±33 mg·L<sup>-1</sup> and 30±10 mg·L<sup>-1</sup> in Periods 1, 2,  
9 3 and 4, respectively. The highest influent TP and PO<sub>4</sub>-P concentrations were observed in  
10 Period 3, while the highest TSS concentration was recorded in Period 4. The results are in line  
11 with existing literature that were reported for typical real municipal wastewater, except for  
12 parameters such as EC, TSS, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> that were measured comparatively higher due to  
13 seawater intrusion in the WWTP of Falconara Marittima [3,5,27].

### 14 **3.2. Start-up period of UASB**

15 The UASB reactor was inoculated with anaerobic sludge (granular sludge TS = 2.72%, VS/TS  
16 = 74%; flocculent sludge TS = 1.42%, VS/TS = 60%) taken from a paper mill WWTP in  
17 Castelfranco Veneto, Italy. The temperature of the UASB was increased gradually to 30°C after  
18 10 days of operation period. The reactor operated for 5 months at 30°C with OLR value of 1.05  
19 ± 0.4 kgCOD·m<sup>3</sup><sup>-1</sup>·d<sup>-1</sup>. The flow rate was maintained between at 3.38 ± 0.6 L·h<sup>-1</sup>. Meanwhile, α  
20 was between 0.12 and 0.43, which indicated a stable biological process. The average biogas  
21 production was 0.39 ± 0.2 Lbiogas·d<sup>-1</sup>. The COD and TSS removal efficiencies were 63% and  
22 84%, respectively. In addition, 86% of P and N were released during the start-up period of  
23 UASB.

### 24 **3.3. Effect of OLR in the system performance**

1 Following the start-up period, the UASB was first fed with the mixture of municipal wastewater  
2 and methanol (as the external C source) and the OLR was increased to  $2.1 \pm 0.6 \text{ kgCOD m}^{-3}\text{d}^{-1}$   
3  $^1$  (Period 2). The flow rate was maintained at  $2.98 \pm 0.3 \text{ L h}^{-1}$ . The variations in OLR and  $\alpha$   
4 value throughout the operation period are given in **Figure 3a** and **Figure 3b**, respectively.  $\alpha$   
5 value remained almost stable in the beginning of Period 2, and then tended to increase up to  
6 0.65. while 86% P and 88% N were released. The average biogas production increased to  $2.8 \pm$   
7  $1.5 \text{ L biogas d}^{-1}$  with  $33.6\% \pm 10.5\%$  of  $\text{CH}_4$  in Period 2. The COD and TSS removal efficiencies  
8 of the UASB was 70% and 48% respectively while the application of the AnMBR increased  
9 the average COD and TSS removal to 85%, and  $> 99.99\%$ . On the other hand, the release of P  
10 and N in the UASB was 86% P and 88% N and then slightly decreased to 76% and 83% in the  
11 integrated UASB+AnMBR configuration, respectively.

12 In Period 3,  $\alpha$  value was between 0.16 and 0.52 with an average of  $0.29 \pm 0.1$ . The addition of  
13 the fermentation liquid in the influent resulted in a peak in the biogas production; while only  
14 9%  $\text{CH}_4$  was measured indicating excess  $\text{CO}_2$  production via fermentation (**Figure 4**). The  
15 biogas production started to increase gradually together with the  $\text{CH}_4$  content of the biogas.  
16 Hence, up to  $10.25 \text{ L biogas d}^{-1}$  was generated with 51.9% of  $\text{CH}_4$  (average of  $4.11 \pm 3.1$   
17  $\text{L biogas d}^{-1}$  with  $29.7\% \pm 11.8\%$  of  $\text{CH}_4$ ). The addition of fermentation liquid as the external  
18 carbon source increased the biogas production without affecting the overall  $\text{CH}_4$  content of the  
19 biogas. In Period 3, the COD removal efficiencies were 42% and 83% in UASB and  
20 UASB+AnMBR, respectively. The TSS removal efficiencies in UASB and UASB+AnMBR  
21 were 38% and 100%, respectively, while P and N releases were 85% and 75%, respectively.

22 The application of the AnMBR in the study of Gouveia et al. [2] for the treatment of municipal  
23 wastewater under psychrophilic conditions and loading rate of 2 and  $2.5 \text{ kgCOD m}^{-3}\text{d}^{-1}$   
24 resulted in effluent tCOD concentrations of  $100 \text{ mg L}^{-1}$ – $120 \text{ mg L}^{-1}$ . In another study by Wei  
25 et al. [28], a wide range of volumetric OLR ( $0.8$ – $10 \text{ gCOD L}^{-1}\text{d}^{-1}$ ) was tested in AnMBR to treat

1 synthetic municipal wastewater. The results showed that at steady conditions, 98% COD  
2 removal was achieved while the application of high sludge OLR led to high methane production  
3 of over 300 mL·gCOD<sup>-1</sup>. Wijekoon et al. [29] tested the performance of a thermophilic AnMBR  
4 at different OLRs ranging from 5.12 kgCOD·m<sup>3</sup>·d<sup>-1</sup> to 12 kgCOD·m<sup>3</sup>·d<sup>-1</sup>. The authors reported  
5 an average biogas production of 15 L·d<sup>-1</sup>, 20 L·d<sup>-1</sup> and 35 L·d<sup>-1</sup> at OLRs of 5.1 ± 0.1 kgCOD·m<sup>3</sup>·  
6 d<sup>-1</sup>, 8.1 ± 0.3 kgCOD·m<sup>3</sup>·d<sup>-1</sup> and 12.0 ± 0.2 kgCOD·m<sup>3</sup>·d<sup>-1</sup>, respectively, with CH<sub>4</sub> content of  
7 about 55%–65%. In addition, the reactor showed optimum COD removal efficiencies at 8 ± 0.3  
8 kgCOD·m<sup>3</sup>·d<sup>-1</sup> OLR. In a recent study, the highest VFA yield (48.20 ± 1.21 mgVFA·10  
9 mgCOD<sub>feed</sub><sup>-1</sup>) was observed at OLR of 550 mgCOD·L<sup>-1</sup>; however, the authors achieved less VFA  
10 yield at the examined maximum OLR (715 mgCOD·L<sup>-1</sup>), indicating that elevated OLRs can  
11 lead to high VFA production but it is also crucial to optimize operating OLR during the  
12 treatment of low strength wastewater in AnMBR [30]. In high-rate bioreactors such as UASB  
13 and AnMBR, VFAs may not be efficiently converted to methane due to low-retention times  
14 and can accumulate in the reactor and thus can be detected in the effluent [1,31]. In operating  
15 conditions at elevated OLRs, VFAs should be therefore monitored to meet the local standards  
16 for discharge or reuse.

### 17 **3.4. Effect of salinity in the system performance**

18 The impact of high salinity conditions was assessed in Period 4. Chloride concentration during  
19 all operational periods are given in **Figure 5**. The red points mark the membrane cleaning days.  
20  $\alpha$  value was stable around 0.3. Biogas production gradually decreased with increasing Cl<sup>-</sup>  
21 concentrations (see **Figure 4**). Although the biogas production was 1.2-1.3 L·d<sup>-1</sup> during in the  
22 beginning of Period 4 (so-called initial low saline conditions at 200 mgCl<sup>-</sup>·L<sup>-1</sup>), it decreased to  
23 0.13-0.57 L·d<sup>-1</sup> in the presence of 500 mgCl<sup>-</sup>·L<sup>-1</sup>. When the Cl<sup>-</sup> concentration was increased to  
24 1500 mgL<sup>-1</sup> (at the end of Period 4), the CH<sub>4</sub> content in biogas reduced by 27% compared to  
25 the reported values under low saline conditions. The CH<sub>4</sub> content of the biogas was adversely

1 affected (10%-20% in the beginning of Period 4 and 5% at the end) by high saline conditions.  
2 The system almost failed to operate at the maximum examined  $\text{Cl}^-$  concentration (app. 2200  
3  $\text{mg}\cdot\text{L}^{-1}$ ), since the biogas production was  $0.08 \text{ L}\cdot\text{d}^{-1}$  with 3%  $\text{CH}_4$ .  
4 In line with the results of the current study, Aslan et al. [32] demonstrated that the COD removal  
5 significantly decreased at about  $20 \text{ gNa}^+\cdot\text{L}^{-1}$  when treating saline wastewater in a UASB reactor.  
6 Reduced biogas production and COD removal were also reported by Song et al. [12] in an  
7 AnMBR operating under saline conditions  $15 \text{ gNa}^+\cdot\text{L}^{-1}$ . A decrease in biomass production was  
8 observed with the increase of salinity in the AnMBR. A NaCl shock load (increase from 5  
9  $\text{gNaCl}\cdot\text{L}^{-1}$  to  $60 \text{ gNaCl}\cdot\text{L}^{-1}$ ) caused a reduction of COD and TKN removal efficiencies in the  
10 study of Yogalakshmi et al. [17]; COD and TKN removal dropped to 64% and 23% at 60  
11  $\text{gNaCl}\cdot\text{L}^{-1}$ , respectively, while the nitrification was completely inhibited. In the study of Luo et  
12 al [33], a reduction of TOC and  $\text{NH}_4^+\text{-N}$  removals was initially observed with elevated NaCl  
13 loading; however, microbial diversity in saline AnMBR did not change and the adaptation of  
14 microbial community to saline conditions was stated to recover AnMBR biological  
15 performance. In a recent work of Muñoz Sierra et al. [34], the performance of UASB and  
16 AnMBR was evaluated for the treatment of highly-saline phenolic wastewater. The authors  
17 highlighted the superiority of AnMBR over UASB in terms of bioreactor conversion, biomass  
18 characteristics and microbial community under salinity up to  $26 \text{ gNa}^+\cdot\text{L}^{-1}$  due to its greater  
19 probability to maintain functionality and to respond to high salinity. In another study of the  
20 same authors [18], a short-term salinity fluctuation of  $18 \text{ gNa}^+\cdot\text{L}^{-1}$  to  $20 \text{ gNa}^+\cdot\text{L}^{-1}$  did not affect  
21 the long term operation of AnMBR. The results indicated that high saline conditions initially  
22 caused a decrease of the biological performance of AnMBR; however, long term adaptation of  
23 microbial community to saline conditions (i.e. halotolerant or even halophilic microorganisms)  
24 is required with high biomass concentrations for the system to regain its stability.

### 25 **3.5. Effect of OLR and high salinity on membrane operation**

1 The variations in the TMP value is shown in **Figure 6a**. The red points mark the membrane  
2 cleaning days. The TMP of the membrane was stable at around 50 mbar when the system was  
3 operated with methanol addition (Period 2, OLR of  $2 \text{ kgCOD}\cdot\text{m}^3\cdot\text{d}^{-1}$ ), with gas-sparging  
4 condition of 10 seconds on and 120 seconds off. The specific flux normalized at  $20^\circ\text{C}$  was  $175$   
5  $\text{L}\cdot\text{h}^{-1}\cdot\text{m}^2\cdot\text{bar}^{-1}$  and only NaOCl cleaning was necessary after 50 days of operation. (**Figure 6b**)  
6 During the fermentation liquid addition (Period 3, OLR of  $2 \text{ kgCOD}\cdot\text{m}^3\cdot\text{d}^{-1}$ ) the behavior was  
7 first similar to the methanol co-treatment; then the TMP increased gradually after 50 days of  
8 operation and reached to 500 mbar after 100 days of operation. Thus, a more intense chemical  
9 cleaning was applied to restore the initial permeability (citric acid at  $1000 \text{ mg}\cdot\text{L}^{-1}$ ) on day 315.  
10 A significant EPS production was observed when the fermentation liquid was used, leading to  
11 larger formation of the "cake" on the surface of the membrane. This was mainly due to the  
12 fluctuation of the characteristics of the production of fermentation liquid. There was an increase  
13 in EPS concentration from  $52.8 \text{ mgEPS}\cdot\text{L}^{-1}$  in the Period 2 to  $70.8 \text{ mgEPS}\cdot\text{L}^{-1}$  in the Period 3.  
14 The latter decreased the membrane permeability a short time, higher rate pore obstruction and  
15 therefore more intense and frequent cleaning was required.  
16 In Period 4, the TMP remained stable at 12 mbar when the reactors were fed with the  
17 fermentation liquid together with additional NaCl to increase salinity in the system, at the  
18 concentration of  $500 \text{ mgCl}^{-1}\cdot\text{L}^{-1}$ . The average TMP was around 50 mbar, at  $\text{Cl}^{-}$  concentration of  
19  $1500 \text{ mgCl}^{-1}\cdot\text{L}^{-1}$ , caused by the increased filtration resistance following the increased TMP due  
20 to the increased EPS concentration in Period 3. The saline conditions therefore only affected  
21 the initial TMP value and remained constant during the system operation.  
22 The TMP of AnMBRs is highly dependent on the critical flux and the sparging rate together  
23 with other environmental and operating conditions [35]. Furthermore, an initial flux below the  
24 critical flux, prior to the introduction of peak flow, is reported to be advantageous to  
25 permeability recovery [36]. In the study of Muñoz Sierra et al. [18], increased salt concentration



1 was found to affect the TMP negatively (350 mbar at a flux of  $4.0 \text{ Lm}^2\text{h}^{-1}$ ). The deterioration  
2 of membrane filtration performance was attributed to the decrease of biomass particle size when  
3 salinity was increased. The small particle size had a significant influence on the cake layer  
4 compaction that increased the operational values of the filtration resistance. Furthermore,  
5 higher stability of process performances of AnMBR over UASB was reported to overcome high  
6 salinity [34]. Elevated TMP values were also reported by Yurtsever et al. [19] with respect to  
7 high salinity conditions. The salinity induced large molecules as foulants in gel/cake layer, that  
8 may originate from biomass loosely bound EPS. The EPS properties are highly dependent on  
9 the operating OLR [37]; and the OLR increase is often accompanied with high EPS production.

### 10 **3.6. Relationship between process parameters and system performance**

11 PCA was carried out to reveal the relationships between the applied operating conditions (i.e.  
12 OLR, salinity) and system performance in terms of biogas production,  $\text{CH}_4$  content of the  
13 biogas,  $\alpha$  value, TMP and  $J_s$ . The PCA supported our previous discussion regarding the  
14 performance of the UASB+AnMBR system at different periods. OLR was closely grouped  
15 together with the biogas production,  $\text{CH}_4$  content and  $\alpha$  value (**Figure 7**). This cluster showed  
16 the close relationship between these parameters mostly in Period 1 which included only the  
17 UASB operation. The data-points of Period 2 was comparatively more equally distributed  
18 between the parameters; where the negative impacts of OLR on TMP were clearly reflected in  
19 our data especially in Period 3. Furthermore, data points of Period 4 were characterized by high  
20 chloride concentrations. The displayed negative correlation between the salinity and TMP  
21 and/or  $J_s$  in Period 4 was due the citric acid cleaning of the membrane following the fouling  
22 occurred in Period 3 that was previously mentioned. TMP value was close to its initial value  
23 right after the citric acid cleaning and although TMP also slightly increased Period 4 at high  
24 chloride concentrations (**see E-Supplementary material**), this increase was relatively low  
25 comparing to Period 3.

### 1 3.7. Assessment of the possible re-use of the effluent

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

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

- 12 • Compliance with the minimum requirements. These requirements differ based on four water  
13 quality classes defined according to the type of crop and the irrigation method.
- 14 • Monitoring of recovered water based on minimum test frequency requirements.

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

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

20 The obtained effluent quality was compared against the values reported in **Table 4** (minimum  
21 requirements for reuse). Analyzing the results obtained from this study, the UASB effluent is  
22 not compliant with the limits required both by D.M. 185/03 and by the new EU proposal. The  
23 permeate of the AnMBR appears of higher quality because it is free from TSS and falls into  
24 class A since ultrafiltration guarantees also disinfection and *E. coli* removal. For the restrictions  
25 posed by the D.M. 185/2003 for the reuse of the treated wastewater are mainly related to the

1 high concentration of chloride (High values are due to the salt water intrusion since the plant  
2 is in a coastal area) and the nutrients concentration (N and P), both exceed the limit values. The  
3 high nutrient content in the permeate (TN reaches a maximum value of 60 mgN·L<sup>-1</sup>, TP of 6  
4 mgP·L<sup>-1</sup>), allows a potential reuse in fertigation field.

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

#### 11 **4. Conclusions**

12 Regarding the change of paradigm in the context of circular economy in terms of water,  
13 nutrients and energy; site-specific optimization of an AnMBR is crucial especially in coastal  
14 regions. In this particular study, the addition of fermented cellulosic sludge to raw wastewater  
15 increased the biogas production without affecting the overall CH<sub>4</sub> content of the biogas. In case  
16 of the application of the process in coastal areas, the biogas production decreased by 27% due  
17 to the saline conditions when Cl<sup>-</sup> concentration was up to 1500 mg·L<sup>-1</sup>. Moreover, the CH<sub>4</sub>  
18 content of the biogas was also adversely related to the high saline conditions up to almost null  
19 value for chloride higher than 2000 mg·L<sup>-1</sup>. At Cl<sup>-</sup> concentration less than 1500 mgCl·L<sup>-1</sup>, long  
20 term adaptation of microbial community (i.e. halotolerant or even halophilic microorganisms)  
21 may be required with high biomass concentrations for the system to regain its stability and  
22 recover the bioreactor performance in UASB+SuMBR. Concerning the membrane operation,  
23 the increased salinity resulted only in the increase of initial TMP value where it stayed constant  
24 during the following operating days. The AnMBR effluent fell into class A since ultrafiltration  
25 guarantees also disinfection and *E. coli* removal.

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## 10 **Appendix A. Supplementary data**

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

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1 **Figure Captions**

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

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

7 **Fig. 3.** Variations in a) OLR b)  $\alpha$  value (the ratio of partial alkalinity over total alkalinity) at  
8 different periods of the operational time.

9 **Fig. 4.** Variations in a) Biogas production b) CH<sub>4</sub> content of the biogas at different periods of  
10 the operational time.

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

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

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