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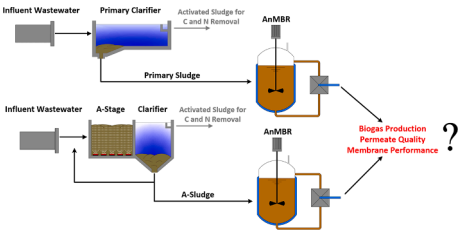
HIGHLIGHTS

- AnMBR technology was used for digestion of primary sludge and A-sludge.
- High permeate quality was obtained, which was free of solids and coliforms.
- Digestion of A-sludge yielded more methane compared to primary sludge.
- The membrane was operated at a higher TMP and it was clogged earlier with A-sludge.

GRAPHICAL ABSTRACT



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ABSTRACT

Energy-rich sludge can be obtained from primary clarifiers preceding biological reactors. Alternatively, the incoming wastewater can be sent to a very-high-loaded activated sludge system, i.e., a so-called A-stage. However, the effects of applying an A-stage instead of a primary clarifier, on the subsequent sludge digestion for long-term operation is still unknown. In this study, biogas production and permeate quality, and filterability characteristics were investigated in a lab-scale anaerobic membrane bioreactor for primary sludge and A-stage sludge (A-sludge) treatment. A higher specific methane yield was obtained from digestion of A-sludge compared to primary sludge. Similarly, specific methanogenic activity was higher when the anaerobic membrane bioreactor was fed with A-sludge compared to primary sludge. Plant-wide mass balance analysis indicated that about 35% of the organic matter in wastewater was recovered as methane by including an A-stage, compared to about 20% with a primary clarifier.

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1. Introduction

Wastewater treatment consumes a significant amount of energy, which amounts up to 1–5% of the total energy consumption in European countries and in the United States (Gude, 2015; Guven et al., 2019). The energy is mainly required for the aerobic conversion of organic matter and ammonium and its consumption accounts for greater than 50% of the total energy consumption in wastewater treatment plants (WWTPs) (Gude, 2015). The energy balance is positively affected by anaerobic sludge digestion, especially primary sludge. The inclusion of a primary clarifier before the biological reactors results in a higher sludge total production compared to the direct treatment of raw wastewater. Typically, about 40% of the organic matter from wastewater is removed during primary settling (for a typical hydraulic retention time (HRT) of 2–3 h), which also significantly reduces the aeration energy demand in subsequent biological reactors. Overall, about 60% of chemical oxygen demand (COD) in the wastewater ends up in the primary sludge and waste activated sludge (WAS), while still 30% of the organic matter is lost through conversion to carbon dioxide (CO_2) and the rest of organics is remained in the effluent (Wan et al., 2016). In order to maximize carbon harvesting and minimize CO_2 losses, innovative systems must be developed, striving for energy-positive WWTPs.

The Adsorption-Bio-oxidation (A-B) process was developed by Bohnke et al. (1997) to harvest and redirect more organics to the sludge stream for anaerobic digestion. An A-stage is a high-rate activated sludge (HRAS) system which is considered as an alternative to primary clarification, which includes a very-high-loaded activated sludge system with an intermediate clarifier. It is operated at short HRTs (0.5–1 h) and solids retention times (SRTs) (1–4 d) under low dissolved oxygen (DO) concentrations (<2 mg/L) (Guven et al., 2019). The soluble organic matters are converted to biomass, which are separated out in the clarifier via flocculation, together with non-degraded colloidal and suspended matters. Extracellular polymeric substances (EPS) act as flocculants in the adsorption process, playing a major role in this process. Therefore, by using an A-stage, more COD in wastewater, i.e., 66%, can be redirected to the sludge stream for anaerobic digestion in comparison with primary clarification, which retains about 40% (Wan et al., 2016). The subsequent B-stage is a bio-oxidation stage, which is operated at long SRTs to guarantee complete nitrification (Guven et al., 219). Therefore, this configuration helps to approach energy self-sufficiency WWTPs.

Anaerobic digestion is widely applied for energy recovery from sludge in WWTPs. Conventional anaerobic digesters for sludge treatment are designed as a completely mixed reactors (HRT = SRT) operated at high SRTs for enhanced solids conversion and to maintain the methanogenic activity in the reactor. Consequently, anaerobic digesters are commonly built with large volumes to ensure sufficient reduction of volatile solids (VS) (Xu et al., 2011). Furthermore, due to the low hydrolysis rates, the organic loading rate (OLR) is maintained low, at 1–3 kg COD/m^3·d (Verstraete and Vandevivere, 1999). Anaerobic membrane bioreactor (AnMBR) is a promising alternative to conventional anaerobic digesters for sludge digestion. AnMBRs are operated at long SRTs independent from HRT by means of physical separation of the membrane. Thus, slow growing methanogenic biomass can be kept longer in the reactor, resulting in enhanced methane production. Moreover, a smaller footprint of the anaerobic reactor can be achieved since the HRT can be controlled by changing the membrane flux. By using microfiltration or ultrafiltration (UF) membranes, a higher quality of permeate can be produced in comparison with the supernatant of conventional anaerobic digesters. Furthermore, nutrients recovery using physicochemical treatment can be more feasible, since the permeate is almost free of solids (Abdelrahman et al., 2021).

The application of AnMBR for sludge treatment has received increased attention over the last few decades (Abdelrahman et al., 2021). Liew Abdullah et al. (2005) studied the effect of OLR on AnMBR treatment performance treating sewage sludge. The biogas yield was improved, increasing from 0.28 to 0.81 m^3/kg COD·d, with OLR increment from 0.1 to 10 kg COD/m^3·d. Cayetano et al. (2020) evaluated comparatively the performance of a lab-scale AnMBR and an anaerobic digester for WAS treatment at different HRTs (10–25 days). It was reported that the AnMBR showed better performance and more stability than the anaerobic digester, in which butyric acid accumulated and methane production decreased. Chen et al. (2021) operated a pilot-scale AnMBR at different SRTs, 30, 40 and 50 days, for WAS digestion. Improvement in digestion efficiency was observed by increasing the SRT, however, solids concentration significantly increased in the reactor, resulting in a sudden increase in transmembrane pressure (TMP). Cheng et al. (2020) aimed to upgrade the methane yield by co-digesting sludge with food waste in an AnMBR. It was reported that the optimum food waste/sewage sludge ratio was 75%:25%, in which methane yield was 0.295 L CH_4 /g COD_{fed} which was 67.7% higher than that of sewage sludge mono-digestion.

Anaerobic digestion of sludge obtained from an A-stage process (A-sludge) has previously been compared to that of WAS and/or sludge obtained from the B-stage (B-sludge) (Trzcinski et al., 2016; Cagnetta et al., 2017). Notably, WAS and B-sludge are originating from reactors operated at long SRTs, resulting in decreased sludge biodegradability (Bolzonella et al., 2005). To the best of authors' knowledge, no study has been conducted to examine the impact of integrating an A-stage, instead of a primary clarifier, on the sludge digestion. Moreover, little is known on the performance of an AnMBR in digesting these sludges. This study is the first to investigate and compare the digestibility of A-sludge and primary sludge in an AnMBR. The treatment performance was assessed in terms of biogas production and organic matter removal efficiency, process stability and permeate quality. The filtration performance of an UF membrane applied for the physical separation of anaerobic sludge was assessed as well. Primary sludge and A-sludge were compared in terms of treatment and filtration performances. Morphological analyses were conducted to have a better understanding of the membrane fouling layer. Finally, a plant-wide COD mass balance analysis was conducted.

2. Materials and methods

2.1. Substrate characteristics

Primary sludge and A-sludge were the substrates in this study. The primary sludge was obtained from a primary clarifier of a full-scale WWTP with a daily capacity of 600,000 m^3. The A-sludge was obtained from the return activated sludge line of a pilot-scale A-stage system. The A-stage is an HRAS system which was operated at DO concentration of 0.5 mg/L, HRT of 75 min and SRT of 0.5 d. The sludge was sieved through a 2 mm mesh screen to remove the coarse particles and was stored at 4 °C. The sludge characteristics are given in Table 1.

2.2. Lab-scale Set-up

The AnMBR consisted of a cylindrical glass reactor (working volume of 7 L), equipped with an external membrane configuration (Fig. 1). The substrate was kept in a refrigerator at 4 °C and mixed by a mechanical mixer before feeding. Substrate was fed continuously with a peristaltic pump. A mechanical mixer was used to mix the sludge inside the reactor. The reactor was equipped with a water jacket for temperature control. The reactor was equipped with pH, temperature, oxidation reduction potential (ORP) and level sensors, and gas meter. TMP was measured by pressure transmitters placed on the inlet, outlet and permeate lines. A Mono™ progressing cavity pump was used for sludge circulation inside the membrane module to achieve a specific cross flow velocity on the membrane surface. A vacuum pump was used to obtain the permeate. A Programmable Logic Controller (PLC) was used to control the pumps and to record the data which are obtained from the sensors. A computer was connected to the PLC for the operation via a control and data acquisition program (SCADA software). A commercial UF Flat Sheet

Table 1
Characterization of two different sludge types.

Parameter	Unit	Primary sludge	A-sludge
Total suspended solids (TSS)	mg/L	11,035 ± 714	9,555 ± 417
Volatile suspended solids (VSS)	mg/L	5,198 ± 311	5,643 ± 231
VSS/TSS	%	47.1 ± 1.4	59.1 ± 0.8
Total solids (TS)	mg/L	12,553 ± 667	13,477 ± 405
Volatile solids (VS)	mg/L	5,853 ± 372	6,616 ± 184
COD	mg/L	10,100 ± 289	10,524 ± 380
Soluble COD (sCOD)	mg/L	1,953 ± 86	2,229 ± 66
Total nitrogen (TN)	mg/L	329 ± 11	609 ± 21
Ammonium- nitrogen (NH_4-N)	mg/L	71 ± 4	216 ± 11
Total phosphorous (TP)	mg/L	53 ± 3	115 ± 5
Dissolved phosphorous (DP)	mg/L	1.66 ± 0.04	22.18 ± 1.73
pH	–	6.5 ± 0.2	6.7 ± 0.2
Conductivity	ms/cm	1.9 ± 0.1	6.5 ± 0.1
Fecal coliform	MPN*/g TS	$2x10^5$ ± $0.70x10^5$	$3.5x10^5$ ± $0.34x10^5$
Total coliform	MPN*/g TS	$3.7x10^5$ ± $0.35x10^5$	$6.5x10^5$ ± $0.32x10^5$
Capillary suction time (CST)	sec	54 ± 3	117 ± 7
Median particle size (d_{50})	μm	33 ± 1	206 ± 5

*MPN: Most probable number.

membrane was used in the experimental study. The membrane was made of polyvinylidene difluoride (PVDF) with a pore size of 0.02 μm and filtration area of 0.012 m^2.

2.3. Inoculum

The lab-scale AnMBR was fed for 1.5 months with primary sludge. The reactor content was then harvested and stored at 4 °C to serve as inoculum for further experiments. The inoculum characteristics are shown in Table 2. The ratio between VS and total solids (TS) was 41.3% with an average TS concentration of 49,795 mg/L.

2.4. Experimental procedure

The inoculum was divided to fill the AnMBR in two separate operations. The AnMBR was firstly fed with primary sludge for 94 days. Then, the AnMBR was emptied and filled again with the inoculum and fed with A-sludge for 109 days. The system was operated for 55 days under stable digestion conditions. Stable conditions were determined when daily variation of biogas production was less than 10% for at least 10 days. All reported average values were calculated based on stable process performance. Temperature was kept around 35 °C, targeting mesophilic digestion. The AnMBR was operated at HRT of 3.33 days and OLR of 3 kg COD/m^3·d. The SRT was kept at 25 days by controlling the daily waste sludge. The membrane flux was increased in three steps till it reached 11 L/m^2·h. The membrane was operated in series of filtration and backwashing cycles. The periods of filtration and backwashing were 190 and 35 sec, respectively. The backwashing was performed by using the permeate. The cross flow velocity in the membrane module was set around 0.1 m/sec by adjusting the recirculation rate at 50 L/min.

Table 2
Inoculum characteristics.

Parameter	Unit	Value ± Standard Deviation
TS	mg/L	49,795 ± 262
VS	mg/L	20,563 ± 244
TSS	mg/L	48,600 ± 566
VSS	mg/L	20,417 ± 212
COD	mg/L	41,268 ± 172
sCOD	mg/L	1,360 ± 11
Alkalinity	mg $CaCO_3$/L	8,188 ± 18
NH_4-N	mg/L	568 ± 11
CST	sec	50.8 ± 1.0
d_{50}	μm	10.4 ± 0.6
Volatile fatty acids (VFA)	mg COD/L	3,942 ± 73
Specific methanogenic activity (SMA)	g CH_4-COD/g VS·d	0.12 ± 0.007

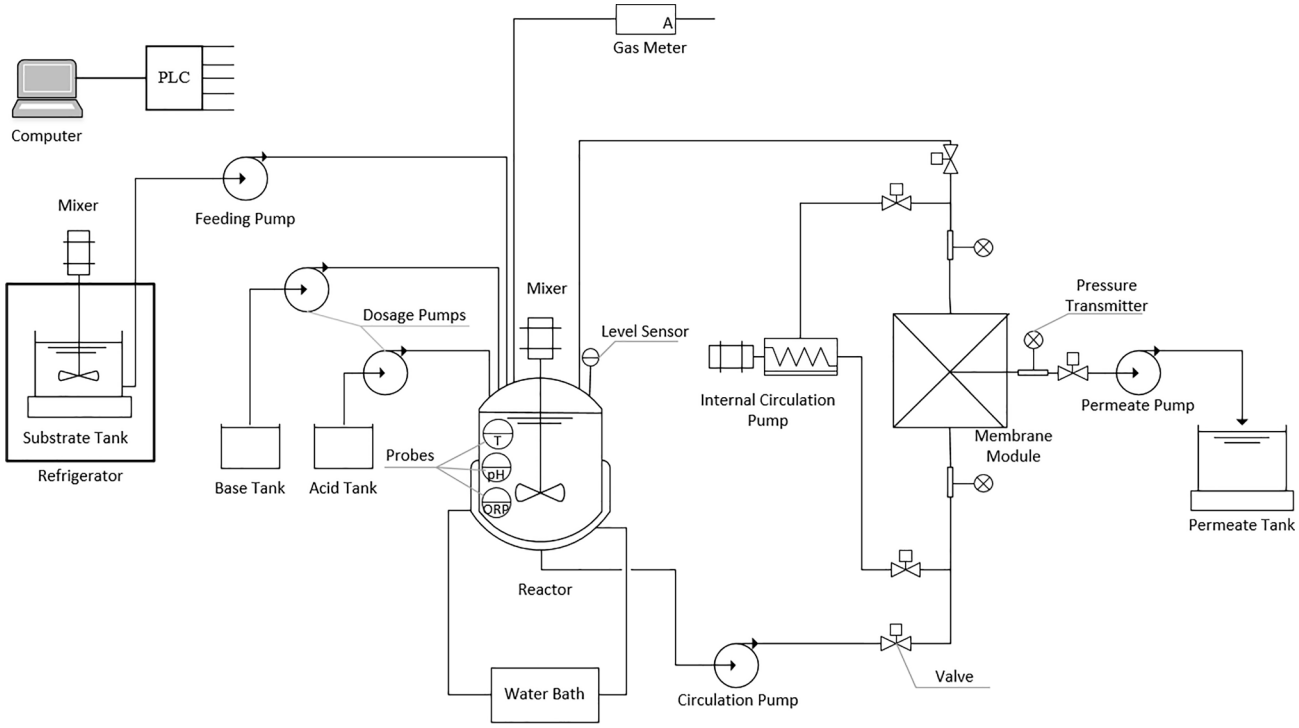

Fig. 1. Schematic diagram of AnMBR set-up.

2.5. Experimental analysis

2.5.1. Analytical methods

TSS, VSS, TS, VS, COD, sCOD, TP, DP, TN, NH_4-N, alkalinity, fecal coliform and total coliform were measured according to APHA (2017). CST measurements were performed by a CST analyzer (Triton Electronics, Type 304 M, UK). The d_{50} values of the anaerobic sludge and substrates were determined by a Mastersizer 2000 (Malvern Instruments, Hydro 2000 MU, UK). The methane content (%CH_4) in biogas was measured via gas chromatography (GC) with flame ionization detector (FID) (Agilent 7890 A, USA). VFA analyses were conducted by using a GC equipped with FID (GC-FID) (Shimadzu, Japan). The Student's *t*-test was performed with a significance level of probability (p-value) of 0.05 by using Microsoft Excel 2016.

The specific methanogenic activity (SMA) of the anaerobic sludge was measured by an Automated Methane Potential Test System II (Bioprocess Control, Sweden). The anaerobic sludge was collected at the end of the operational period. Samples and blanks were analyzed as triplicates in 500 mL bottles (working volume of 400 mL). The bottles were flushed with nitrogen gas prior to the test to get rid of oxygen. Sodium acetate was used as substrate for samples. SMA tests were carried out at 37 °C. The addition of phosphate buffer solution, macronutrients and trace elements was carried out by following the steps mentioned in the study of Ozgun et al. (2015). Sludge VS concentration was set as two-fold of substrate COD concentration.

Anaerobic sludge sample was taken weekly to measure soluble microbial products (SMP) and bound EPS, including tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS). The samples were filtered through 0.45-μm filters to measure the SMP. Bound EPS was extracted by heat extraction method described in the study of Kinyua et al. (2017). Carbohydrate and protein fractions of SMP, LB-EPS and TB-EPS were determined following the procedures of Dubois et al. (1956) and modified Lowry method (Frølund et al., 1995), respectively.

2.5.2. Morphological analyses

The membrane samples were taken at the end of the operational period. Then, the samples were dried at 4 °C prior to conducting the morphological analyses. The surface morphology of membrane sample was visualized by environmental scanning electron microscopy (ESEM) (Thermo Fisher Scientific Inc., FEI Quanta FEG 250 ESEM, UK). Organic materials on the surface of the membrane were identified by using Fourier transform infrared spectroscopy (FTIR) (Perkin-Elmer Inc., Spectrum 100 spectrometer, USA). Confocal laser scanning microscopy (CLSM) was used for visualizing biofilms attached to the membranes after staining the samples with Live/Dead BacLight™ Bacterial viability kit (Thermofisher Scientific, USA). The preparations of the samples were performed as described by Isik et al. (2019).

2.6. Mass balance calculations

A COD mass balance was set up to evaluate the digestibility of the sludge in the AnMBR. The mass balance was conducted based on Eq. (1):.

$$\begin{aligned} Q_{Influent}(L/d) \times COD_{Influent}(g/L) = {} & Q_{Permeate}(L/d) \\ & \times COD_{Permeate}(g/L) + Q_{Biogas}(L/d) \\ & \times P_{Methane}(\%) \\ & \times \frac{1}{0.35\,L\,methane/g\,COD} + Q_{Waste\ Sludge}(L/d) \\ & \times COD_{Waste\ Sludge}(g/L) + Q_{Permeate}(L/d) \\ & \times C_{Dissolved\ Methane}\left(L_{Methane}/L_{Liquid}\right) \\ & \times \frac{1}{0.35\,L\,methane/g\,COD} \end{aligned} \quad (1)$$

Where $Q_{Influent}$, $Q_{Permeate}$, Q_{Biogas} and $Q_{Waste\ sludge}$ are the flow rates of influent, permeate, biogas and sludge wasting, respectively; $COD_{influent}$, $COD_{Permeate}$ and $COD_{Waste\ Sludge}$ are the COD concentrations of influent, permeate and sludge wasting, respectively; $P_{Methane}$ is the methane content in biogas; $C_{Dissolved\ Methane}$ is dissolved methane content in liquid, which was estimated based on Eq. (2):

$$C_{Dissolved\ Methane}\left(L_{Methane}/L_{Liquid}\right) = \frac{P_{Methane}\ (\%)\ \times\ P\ (atm)\ \times\ V_c\ (L/mole)\ \times N_w\ (mole/L)}{H\ (atm/mole\ fraction)} \quad (2)$$

Where, $P_{Methane}$ is the methane content in biogas; P is the pressure (1 atm); V_C is the corrected volume of 1 mol of gas at 35 °C (25.27 L/mole); H is Henry's law constant of methane at 35 °C (4.845×10^4 atm/mole fraction); N_W is number of water moles contained in 1 L solution (55.6 mol/L).

3. Results and discussion

3.1. Treatment performance

3.1.1. Biogas production and organic matter removal efficiency

The average biogas production rate during the digestion of primary sludge and A-sludge was 5908 ± 352 and 5486 ± 238 mL/day, respectively (Fig. 2a, b). While the biogas production was higher for primary sludge, the methane content in the biogas was higher for A-sludge (73%) than for primary sludge (62%). The average methane yields of primary sludge and A-sludge were 0.173 ± 0.012 and 0.182 ± 0.009 mL CH_4/ g COD_{fed}, respectively (p-value = 0.047). The higher methane yield for A-sludge may be related to its higher protein content, which can be observed from TN concentration in Table 1. It has indeed been previously reported that the digestion of protein could yield a higher methane content in biogas in comparison with the digestion of carbohydrates (Hu et al., 2020), which could represent about half of the organic content present in the primary sludge (Guo et al., 2020). The average COD concentrations in the AnMBR were similar during feeding with primary sludge and A-sludge (34,656 ± 2,637 and 33,276 ± 1,173 mg/l, respectively). The permeate COD concentration decreased during the start-up period till it reached an average concentration of 440 ± 151 and 281 ± 51 mg/L with removal efficiencies of 95.6 ± 1.5 and 97.3 ± 0.5 % for treatment of primary sludge and A-sludge, respectively (Fig. 2c, d). The high COD removal efficiency of the AnMBR can be related with complete retention of suspended solids by the membrane. Similar findings were reported by Cheng et al. (2021) in which more

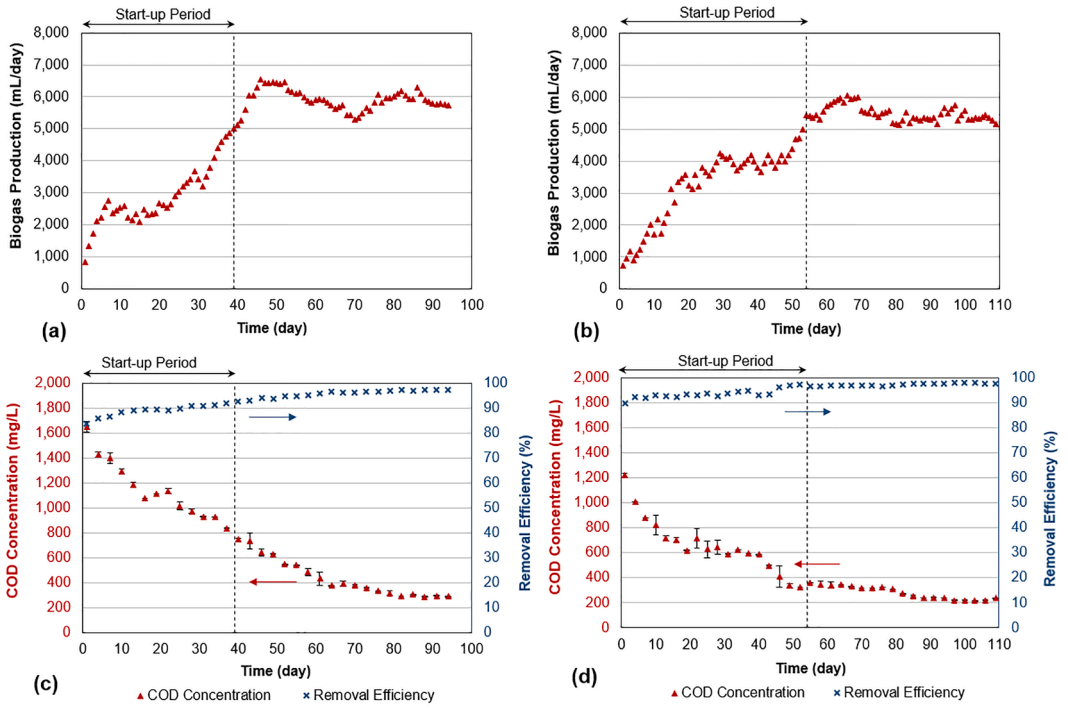

Fig. 2. Biogas production rate (top) and permeate COD concentration (bottom) during the digestion of primary sludge (left: (a),(c)) and A-sludge (right: (b), (d)).

than 99.3% COD removal could be achieved by AnMBR for sludge treatment.

Methanogensis, unlike acidogenesis, is a rate-limiting step in anaerobic digestion process, therefore, SMA is more important than the bacterial degradation of organic matter for primary and A-sludge. Since approximately 70% of COD is converted to methane by the acetoclastic methanogens under mesophilic conditions (Amani et al., 2010), sodium acetate was added as substrate for the SMA tests. Thus, a higher methane

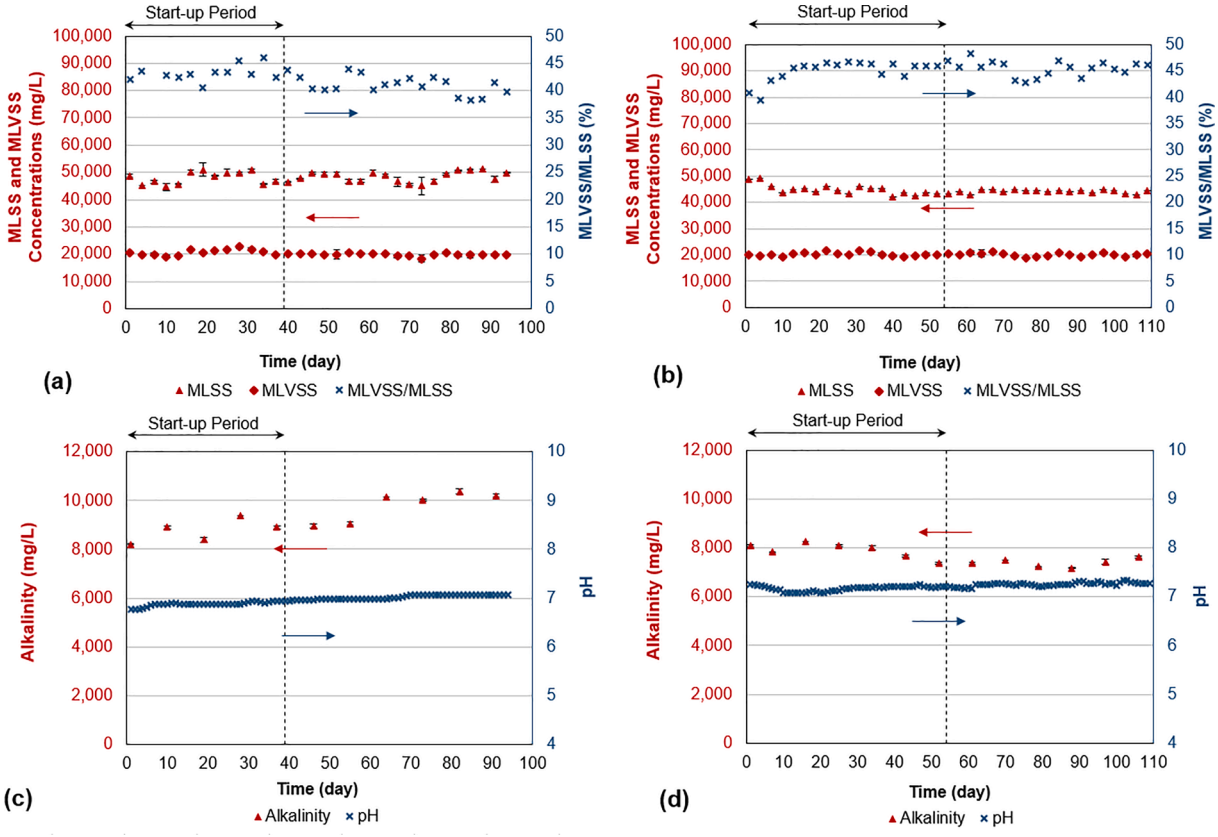

Fig. 3. MLSS and MLVSS concentrations, and MLVSS/MLSS ratio in the AnMBR fed with: (a) primary sludge, (b) A-sludge; pH and alkalinity in the AnMBR fed with: (c) primary sludge, (d) A-sludge.

production rate corresponds to a higher acetoclastic methanogenic activity. The average SMA of the anaerobic sludge fed with primary sludge was 0.13 ± 0.01 g CH_4-COD/g VS·d, which was similar to the SMA of the inoculum. The average SMA of anaerobic sludge fed with A-sludge was 0.19 ± 0.01 g CH_4-COD/g VS·d, which implied an improvement in acetoclastic methanogenesis activity.

3.1.2. Process stability

Solids-liquid separation by the membrane allowed decoupling of HRT and SRT, (Chen et al., 2019), resulting in a high active biomass concentration in the reactor. This was reflected by high suspended solids concentrations in the AnMBR (Fig. 3a, b). The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were quite stable which were resulted from wasting sludge daily to keep the SRT at 25 days. The concentrations of MLSS and MLVSS were similar for primary sludge (48,329 ± 1,851 and 19,824 ± 502 mg/L, respectively) and A-sludge (44,089 ± 633 and 19,964 ± 685 mg/L, respectively). The MLVSS/MLSS ratio was somewhat lower in the AnMBR fed with primary sludge (MLVSS/MLSS = 41 ± 2 %) compared to the one fed with A-sludge (MLVSS/MLSS = 45 ± 1 %), which was due to the lower VSS/TSS ratio of primary sludge compared to A-sludge (Table 1). Thanks to the membrane, the effluent of the AnMBR was almost free of solids, with TSS concentration and turbidity of less than 38 mg/L and 15 NTU, respectively.

VFA, alkalinity and pH are typical indicators used to evaluate the stability of a digester (Cook et al., 2017). The average VFA concentrations in the AnMBR were 426 ± 43 and 573 ± 117 mg/l during feeding with primary sludge and A-sludge, respectively. VFA concentrations lower than 1500–3000 mg/L are recommended for a stable digester operation (Wu et al., 2019). Acetate and propionate were the dominant VFAs, with acetate amounting 67% of the total VFAs in the AnMBR fed with primary sludge. When feeding with A-sludge, 83% of the total VFAs was acetate. The pH was stable and around neutrality during the whole operational period (Fig. 3c, d). The average alkalinity was 9792 ± 570 and 7388 ± 154 mg $CaCO_3$/L in AnMBR fed with primary sludge and A-sludge, respectively. Stable digesters usually have an alkalinity exceeding 2000 mg $CaCO_3$/L (Cook et al., 2017). VFA to alkalinity ratio in the AnMBR was below 0.08 in both sludges. Liu et al. (2012) reported that the anaerobic process is considered stable and there is no risk of VFA accumulation when VFA to alkalinity ratio is less than 0.3. The average pH and ORP were 7.02 ± 0.04 and −462.5 ± 5.4 mV, respectively, in the AnMBR fed with primary sludge. The average pH and ORP in the AnMBR fed with A-sludge were 7.25 ± 0.04 and −482.3 ± 4.7 mV, respectively. The optimum pH and ORP for methanogens are between 7.0 and 8.0 and below −300 mV, respectively (Amani et al., 2010). Overall, the anaerobic system was highly stable in this study.

3.1.3. Permeate quality – Implications for post-treatment

The average TN concentration in the permeate of the AnMBR fed with primary sludge was 156.4 ± 4.8 mg/L, reaching an average TN removal efficiency of 52.5%. Higher TN concentration was observed in the permeate of the AnMBR fed with A-sludge, which was 494.9 ± 12.3 mg/L with an average removal efficiency of 18.8%. Less TN removal could be explained by higher TN (protein) concentration in the A-sludge, which produced NH_4-N during the digestion (protein hydrolysis). Since NH_4-N is soluble, it could pass through the membrane, causing an increase in TN concentration in the permeate. NH_4-N represented 86.3 and 84.8% of TN in permeate of AnMBR fed with primary sludge and A-sludge, respectively. Kanai et al. (2010) stated that the operation of a full-scale AnMBR was very stable because ammonia was washed out by membrane filtration and no inhibition was observed. Ammonia can be removed by partial nitritation-Anammox technology since the permeate had low COD/nitrogen ratio, as 2.8 ± 1.0 and 0.55 ± 0.1 in the case of primary sludge and A-sludge digestion, respectively, which is favorable for Anammox bacteria (Molinuevo et al., 2009). High TP removal efficiency was achieved by the AnMBR fed with primary sludge and A-sludge, with an average of 97.3 and 82.2%, respectively. The lower TP removal efficiency with A-sludge digestion was related with higher DP concentration in A-sludge compared to primary sludge.

No total and fecal coliforms were detected in the permeates, which highlights the effect of membrane filtration on the effluent quality. Similar observations were found in the literature in which pilot-scale AnMBR produced a permeate free of fecal coliforms (Dagnew et al., 2010). Based on the guidelines of US environmental protection agency, the reclaimed water should not contain fecal coliforms if it will be used for irrigation of food crops (USEPA, 2012). Thus, from the hygienic point of view, the permeate has the potential to be directly used for agricultural purposes.

3.2. Filtration performance

3.2.1. Soluble microbial products and extracellular polymeric substances

The SMP and bound EPS concentrations in the AnMBR are given in Table 3. SMP and bound EPS are considered as the origin of organic membrane fouling and they play a key function in fouling (Lin et al., 2014). Protein concentration in SMP was found higher with primary sludge, whereas carbohydrates concentration was higher with A-sludge. Trussell et al. (2006) reported that membrane fouling was well-correlated with the carbohydrates fraction of SMP. For both sludges, protein concentration was higher than carbohydrates concentration in EPS, which can be related to large quantities of exoenzymes in sludge flocs (Frølund et al., 1995). Total bound EPS was higher with A-sludge, which was mainly because of the increase in LB-EPS concentration. LB-EPS was reported to be significantly associated with membrane fouling and increase in membrane resistance more than TB-EPS (Wang et al., 2009). TB-EPS concentration of each sludge was quite similar. Protein/carbohydrates ratio was found higher in LB-EPS compared to TB-EPS. Teng et al. (2020) reported that, after a series of characterizations, LB-EPS had higher protein/carbohydrates and hydrophilicity than TB-EPS.

3.2.2. Particle size distribution and capillary suction time

Particle size distribution (PSD) has a significant effect on membrane fouling, since small flocs more easily deposit on membrane surface (Lin et al., 2010). The average d_{50} of the anaerobic sludge during primary sludge and A-sludge digestion were found as 7.75 ± 0.53 and 10.99 ± 0.42 μm, respectively (see e-supplementary materials). Higher d_{50} during A-sludge digestion could be related to higher d_{50} of A-sludge and higher EPS, especially LB-EPS. Ersahin et al. (2014) reported a reduction in particle size when EPS concentration decreased in the bulk sludge. CST is a parameter to assess dewaterability and filterability of the sludge. Besides, it is used as an indicator parameter to evaluate membrane fouling potential. The average CST of anaerobic sludge for primary sludge and A-sludge digestion was measured as 87 ± 4 sec and 293

Table 3
SMP and EPS compositions in the AnMBR fed with primary sludge and A-sludge.

Parameter	Unit	Primary Sludge	A-sludge
SMP			
Protein	mg/g VSS	15.9 ± 3.1	8.9 ± 2.4
Carbohydrates	mg/g VSS	3.7 ± 0.7	4.6 ± 1.0
Protein/Carbohydrates ratio	–	4.3 ± 0.2	1.9 ± 0.1
LB-EPS			
Protein	mg/g VSS	8.7 ± 1.0	12.9 ± 2.8
Carbohydrates	mg/g VSS	1.8 ± 0.3	3.6 ± 0.1
Protein/Carbohydrates ratio	–	4.9 ± 1.0	3.6 ± 0.7
TB-EPS			
Protein	mg/g VSS	16.2 ± 1.9	16.4 ± 1.5
Carbohydrates	mg/g VSS	4.2 ± 0.2	4.9 ± 0.04
Protein/Carbohydrates ratio	–	3.8 ± 0.6	3.4 ± 0.3
Total bound EPS			
Protein	mg/g VSS	24.9 ± 2.5	29.4 ± 4.3
Carbohydrates	mg/g VSS	6.1 ± 0.4	8.4 ± 0.03
Protein/Carbohydrates Ratio	–	4.2 ± 0.7	3.5 ± 0.5

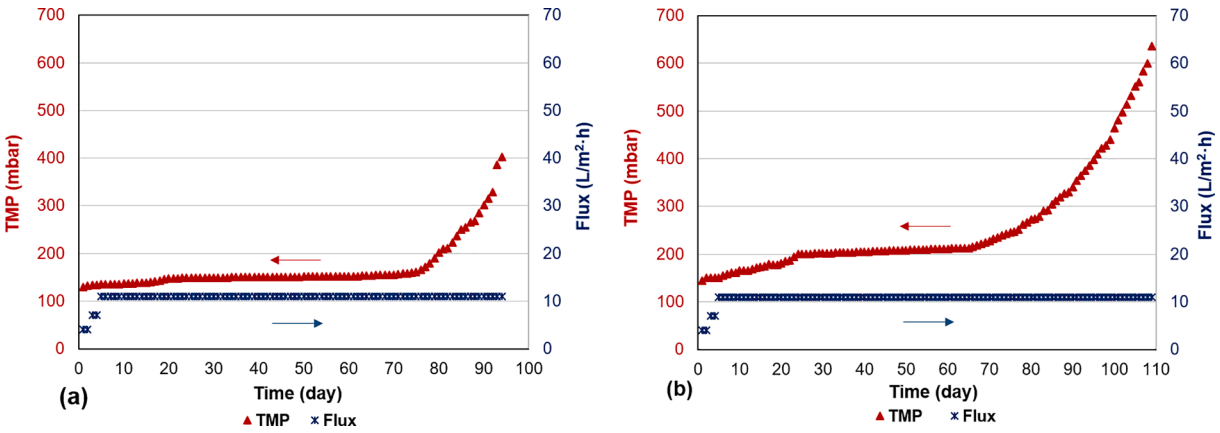

Fig. 4. TMP profile in the AnMBR fed with: (a) primary sludge, (b) A-sludge.

± 11 sec, respectively. The higher CST might be related to the increase in EPS concentration. Sahinkaya et al. (2018) reported that there was a direct relationship between CST and polymeric substances like EPS, where high CST can be related with high EPS concentrations.

3.2.3. Transmembrane pressure and filtration resistance

The flux was increased gradually in two steps till it reached 11 L/m^2·h in order not to have a rapid fouling and sudden increase in TMP (Fig. 4). The membrane was operated continuously without any need for physical and/or chemical cleaning by applying filtration and backwash cycles, as well as a cross-flow shear force. Since the AnMBR fed with A-sludge was operated longer, the average of TMP was calculated based on only 94 days. The average TMPs were 171 ± 53 mbar and 223 ± 51 mbar during digestion of primary sludge and A-sludge, respectively. The average increase rates of TMP were around 3.0 and 4.6 mbar/d for AnMBR fed with primary sludge and A-sludge, respectively. The membrane was operated at a higher TMP and it was clogged earlier with A-sludge, which was correlated to the higher EPS concentration and longer CST of the sludge. Arabi and Nakhla (2008) stated that a higher hydrophobic protein concentration in EPS caused a higher EPS attachment on membrane surface, reducing the membrane permeability. This finding is consistent with this study since protein concentration in EPS was higher with A-sludge. The average filtration resistances were found as $8.0\times10^{12} \pm 2.5 \times10^{12}$ and $1.2\times10^{13} \pm 3.8\times10^{12}$ m^{-1} with primary sludge and A-sludge digestion, respectively.

3.3. Morphological analyses

The surface of the virgin membrane and cake layer at the end of operation were imaged by ESEM (see e-supplementary materials). Crystal-like (inorganic) materials can be seen on both cake layers. Different studies reported that inorganic particles could accumulate on the membrane surface, leading to a rough surface of the cake layer, which is called “mineral scale” (Guo et al., 2012; Villain et al., 2014). Potts et al. (1981) found that carbonate, silica, calcium, sulfate, magnesium, and iron were the main inorganic substances causing membrane fouling. Meanwhile, in this study, the cake layer showed more accumulation on the membrane surface with A-sludge digestion. This accumulation might be related with higher EPS, which caused the higher increase in TMP. Niu et al. (2020) reported that the formation of a compact fouling layer and increase in membrane filtration resistance might be caused by intact microbial cells colonizing in bacteria-EPS clusters, which attached with inorganic particles, and resulted in filled spaces among the biopolymer.

FTIR spectra curves had similar peaks for both cake layers, which suggests that the cake layers had similar functional groups (see e-supplementary materials). The observed peaks at 3288 and 3280 cm^{-1} in the spectrum showed stretching of the O–H bonds in the structure of polysaccharides (Isik et al., 2019). The peaks at 2919 and 2917 cm^{-1} corresponded with aliphatic C–H stretching from polysaccharides (Gao et al. 2011). The secondary structure of protein was indicated by the presence of amides groups. Peaks at 1633 and 1634 cm^{-1} represented amides I (stretching of C=O and C–N bonds). Amides II (deformation of N–H and C=N bonds) corresponded to peaks at 1536 and 1538 cm^{-1} (Isik et al., 2020). Peaks at 1416, 1453 and 1236 cm^{-1} indicated the presence of amides III (C–N stretching) (Wang et al., 2009; Ersahin et al., 2016). The peak observed at 1007 and 1031 cm^{-1} presented the symmetric and asymmetric C=O stretch (at 1000–1200 cm^{-1}) that belong to polysaccharides or polysaccharides-like substances (Ersahin et al., 2016). Peaks at <1000 cm^{-1} (fingerprint region) could coincide to phosphate and sulfate groups, which are functional groups in nucleic acids (Gao et al., 2011). These results exhibited the existence of polysaccharides-like and protein-like substances in the cake layers, which was expected since SMP and EPS likely accumulated on the surface of the membrane.

Bacteria accumulation, live and dead ones, on membrane surfaces was imaged by using CLSM (see e-supplementary materials). In both cake layer, live and dead cells were observed on membrane surfaces. However, a higher amount of dead cells could be observed on the cake layer of the AnMBR fed with A-sludge, which could be associated with existence of more aerobic biomass in A-sludge, which accumulated on the membrane surface.

3.4. Mass balance

Primary clarifier can recover only 40% of wastewater COD in the sludge, while A-stage can recover 66% of wastewater COD (Wan et al., 2016). Thus, based on the COD mass balance of the AnMBR, it can be concluded that an integration of A-stage with an AnMBR can recover around 34.5% of the wastewater COD into methane gas (Fig. 5). While only 19.9% of wastewater COD can be converted into methane gas if a primary clarifier and an AnMBR are used. Dissolved methane accounted for around 0.2% and 0.4% of the wastewater COD with primary clarifier and A-stage integration, respectively. The dissolved methane can be recovered by innovative technologies such as membrane contactors with negligible energy requirements (Velasco et al., 2021). Recovery of methane in membrane contactors can be more efficient with lower hydraulic flow, since the increased retention time of a liquid in membrane module allows longer time period for methane transfer (Li et al., 2021). Thus, dissolved methane recovery from the permeate could be feasible considering that the sludge is produced at low flow rates.

4. Conclusions

In this study, primary and A-sludge digestion was investigated in an

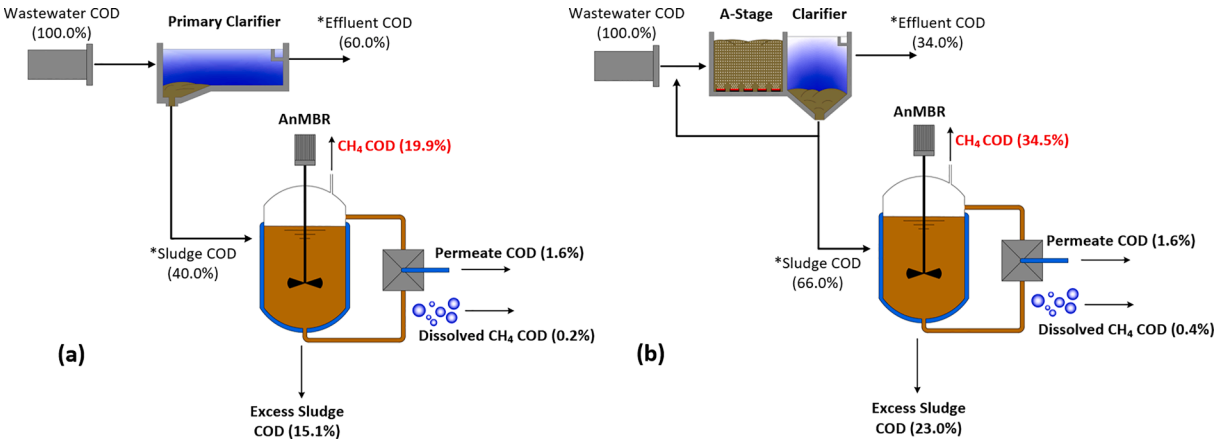

Fig. 5. COD mass balance of AnMBR fed with: (a) primary sludge, (b) A-sludge. (*) . adopted from Wan et al. (2016)

AnMBR to elucidate the effects of applying A-stage instead of primary clarifier on sludge digestion as basis for energy-positive WWTPs. Anaerobic digestion of A-sludge yielded more methane and improved methanogenic activity in the AnMBR. A higher EPS concentration was observed during digestion of A-sludge, which accumulated on the surface of membrane and caused an increase in TMP. On a plant-wide level, integration of A-stage increased the amount of organic matter (COD) recovered from wastewater in form of methane gas by about 15% compared to WWTP configuration with primary clarifiers.

CRediT authorship contribution statement

Amr Mustafa Abdelrahman: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Muhammed Furkan Aras:** Investigation. **Busra Cicekalan:** Investigation, Writing – original draft. **Malhun Fakioglu:** Investigation, Writing – original draft. **Seyma Cingoz:** Investigation, Funding acquisition. **Safak Basa:** Investigation, Funding acquisition. **Huseyin Guven:** Conceptualization, Methodology, Writing – review & editing, Project administration. **Hale Ozgun:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **Izzet Ozturk:** Writing – review & editing, Project administration. **Ismail Koyuncu:** Resources, Writing – review & editing. **Jules B. van Lier:** Conceptualization, Writing – review & editing, Supervision. **Eveline I.P. Volcke:** Conceptualization, Writing – review & editing, Supervision. **Mustafa Evren Ersahin:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.126965.

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