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DOI
10.1016/j.biortech.2024.131822

Publication date
2024

Document Version
Final published version

Published in
Bioresource Technology

Bioresource Technology 417 (2025) 131822

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Mesophilic versus thermophilic digestion of sludge in anaerobic membrane bioreactors

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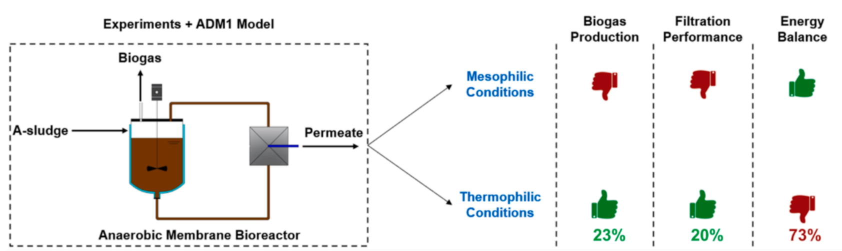
HIGHLIGHTS

- AnMBR was used for A-sludge digestion at mesophilic and thermophilic conditions.
- Higher biogas production was observed in thermophilic AnMBR.
- Membrane was fouled faster in mesophilic AnMBR.
- Net energy recovery of mesophilic AnMBR was three times higher.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Anaerobic digestion
A-sludge
Energy balance
High rate activated sludge
Modeling
Temperature effect

ABSTRACT

Energy-efficient wastewater treatment plants (WWTPs) utilize systems like high-rate activated sludge (A-stage) system to redirect organics from wastewater are redirected into energy-rich sludge (A-sludge). Anaerobic membrane bioreactors (AnMBRs) offer lower footprint and higher effluent quality compared to conventional digesters. In this study, the biological treatment and the filtration performances of AnMBRs for A-sludge digestion under mesophilic and thermophilic conditions were comparatively evaluated through lab-scale experiments, mass balancing and dynamic modeling. Under thermophilic conditions, a higher COD fraction of the influent sludge was converted into methane gas than under mesophilic conditions (65% versus 57%). The energy balance indicated that the surplus energy recovery under thermophilic conditions was less than the additional energy required for heating the AnMBR, resulting in a more than three-fold higher net energy recovery under mesophilic conditions. Therefore, operating an AnMBR for sludge digestion under mesophilic conditions has a higher potential to improve the energy balance in WWTPs.

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https://doi.org/10.1016/j.biortech.2024.131822
Received 5 September 2024; Received in revised form 3 November 2024; Accepted 12 November 2024
Available online 16 November 2024

1. Introduction

Anaerobic digestion is the most commonly applied method for sludge treatment to improve energy recovery in wastewater treatment plants (WWTPs). The organics are converted into biogas in the absence of oxygen through four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Appels et al., 2008). Anaerobic digesters are operated at either mesophilic (35 °C) or thermophilic (55 °C) conditions, which are suitable for the growth of anaerobic microbial consortium. Treatment of sludge under thermophilic conditions provides several advantages over mesophilic ones. Biochemical reactions are accelerated under thermophilic conditions, which result in improvement in organic degradation efficiency or lower applicable retention times. Thus, higher biogas can be produced from a thermophilic digester operated at a similar solids retention time (SRT) to a mesophilic one. In addition, more pathogens are destructed, and less foam is formed in the thermophilic digesters. The low viscosity of sludge in thermophilic conditions is another advantage which makes the sludge easier to mix, transport and/or pump in addition to improving its dewaterability properties. However, the treatment of sludge under thermophilic conditions has some disadvantages, which may affect its feasibility to be used in WWTPs. Thermophilic digesters require higher energy for heating compared to the mesophilic ones (Kardos et al., 2011; Gebreeyessus, and Jenicek, 2016). Anaerobic digesters are constructed with a large footprint to allow the slow-growing microorganisms to have enough time (20–30 days) for organics degradation. For this purpose, the digesters are designed at high SRTs, which is identical to hydraulic retention time (HRT) (Pileggi and Parker, 2017; Ersahin, 2018).

Anaerobic membrane bioreactor (AnMBR) technology is an innovative alternative for anaerobic digesters to have reactors with a lower footprint (Abdelrahman et al., 2021). Integration of a membrane filtration device with an anaerobic digester allows decoupling the HRT and SRT. Therefore, the reactor volume can be reduced by decreasing the HRT, which is governed by the membrane flux. Besides, the membrane ensures that the microorganisms and solids are retained inside the digester. Thus, SRT can be increased by controlling sludge wasting discharge, and more organics can be degraded leading to higher biogas production at the same reactor volume (Liao et al., 2006). Furthermore, AnMBR outperforms conventional anaerobic digesters in terms of effluent quality. Thanks to the membrane, solids-free permeate can be obtained, which makes the recovery of nutrients more feasible than the supernatant of conventional digesters. In addition, since the HRT of AnMBR is lower, some soluble matter (inhibitors) such as ammonium could pass through the membrane, providing more stable conditions for the anaerobic process (Kanai et al., 2010; Abdelrahman et al., 2022). However, membrane fouling limits the AnMBR application to be applied as a full-scale system in WWTPs due to the requirements for frequent membrane cleaning and membrane replacement (Ozgun et al., 2013). Thus, more strategies needed to be investigated to obtain long-term operation without serious membrane fouling, which includes optimization of operational conditions.

In the last few decades, the research interest has been increasing towards sludge treatment by AnMBR technology (Abdelrahman et al., 2021). However, only few studies have focused on the effect of temperature on AnMBR performance and membrane fouling (Meabe et al., 2013; Ozgun et al., 2015; Kim et al., 2017; Pileggi and Parker, 2017). Pileggi and Parker (2017) compared the performance of a pilot-scale AnMBR and conventional anaerobic digester at different temperatures (25, 35 and 55 °C). The AnMBR provided higher volatile solids (VS) destruction and higher methane production compared to the anaerobic digester at all tested temperatures. In addition, methane yield was correlated to the temperature, in which methane yields in AnMBR were 0.19, 0.28 and 0.34 $Nm^3/kg\ VS_{fed}$ at 25, 35 and 55 °C, respectively. Meabe et al. (2013) reported similar biogas generation from mesophilic and thermophilic AnMBRs, however they found that temperature had a significant effect on sludge characteristics. Particle sizes in sludge were smaller (higher amount of soluble organics) in the thermophilic AnMBR, which led to a decrease in permeate quality. Besides, lower viscosity was reported in the thermophilic AnMBR, which provided better filtration performance (Meabe et al., 2013). Kim et al. (2017) reported an increased methane yield, from around 0.48 to 0.57 $m^3/kg\ VS_{fed}$, when increasing the temperature from 35 to 55 °C in the methanogenic reactor of a two-stage AnMBR.

Integration of novel configurations, such as A-stage for organic capture from wastewater has the potential to increase the energy recovered in the WWTP. A-stage is a high-rate activated sludge system, which is an alternative to primary clarifier. It is operated at short HRT (<1 h) and SRT (<2 days), and low dissolved oxygen (DO) concentration (<1 mg/L). Biosorption and bio-flocculation are the main mechanisms for organics redirection into sludge stream (Guven et al., 2019). Abdelrahman et al. (2023) indicated that using A-stage resulted in redirection of more than 50 % of the organics in the wastewater into the sludge (A-sludge), compared to 31 % for primary clarification. It was also reported that 40 % of the organics in A-sludge were proteins, besides, methane yield of A-sludge digestion (333 mL CH_4/g VS) was close to that of primary sludge (346 mL CH_4/g VS), which resulted in improving the overall energy balance of the WWTP. Liang et al. (2022) reported that integration of A-stage for wastewater treatment and AnMBR for sludge treatment resulted in a 30 % overall COD recovery in the form of methane. Similarly, through plant-wide analysis, Abdelrahman et al. (2022) reported that integration of an A-stage system with AnMBR can recover around 35 % of organics embedded in municipal wastewater into methane, compared to only 20 % by integration of primary clarifier with AnMBR. This finding is a step towards achieving positive-energy WWTPs. However, very limited studies have focused on digestion of sludge generated from A-stage in AnMBR.

The aim of this study was to investigate the digestion of sludge obtained from an A-stage (A-sludge) in a lab-scale AnMBR at mesophilic and thermophilic conditions. Both the biological treatment and the filtration performance of the AnMBR were evaluated. Morphological analyses were conducted to get insight into the fouling layer at each condition. A chemical oxygen demand (COD) mass balance was carried out over the AnMBR at both conditions to have a better understanding of the fate of COD in the AnMBR. The anaerobic digestion model 1 (ADM1) was used to dynamically simulate the AnMBR process. Finally, energy balances were set up to evaluate the sludge digestion in mesophilic and thermophilic AnMBRs.

2. Materials and Methods

2.1. Experimental set-up

The lab-scale AnMBR in this study consisted of a cylindrical glass reactor coupled to an external membrane unit (Fig. 1). The working volume of the reactor was 7 L. The temperature of the reactor was controlled through a water jacket. Temperature and pH probes, oxidation–reduction potential (ORP), level, and pressure sensors were fitted inside the bioreactor. A gas meter was connected to the reactor. The transmembrane pressure (TMP) was measured via pressure transmitters (Jumo Midas, Germany) located on the inlet, outlet, and permeate lines. Peristaltic pumps (Lead fluid BT101S, China) were used for substrate feeding, sludge circulation and permeate extraction. A progressing cavity pump (internal circulation pump) ((Seepex BN 5-6L, Germany) was connected to the membrane module to obtain high crossflow velocity (around 0.1 m/sec) on the membrane surface. A commercial UF flat sheet membrane (PHILOS Co., Ltd., Korea) was used for physical separation and to produce permeate. The membrane was made of polyvinylidene difluoride (PVDF) with a pore size of 0.02 µm. The membrane filtration area was 0.024 m^2. All pumps and sensors were connected to a computer, equipped with supervisory control and data acquisition (SCADA) software for controlling the pump operation via programable logic controller (PLC) and for recording the sensor data

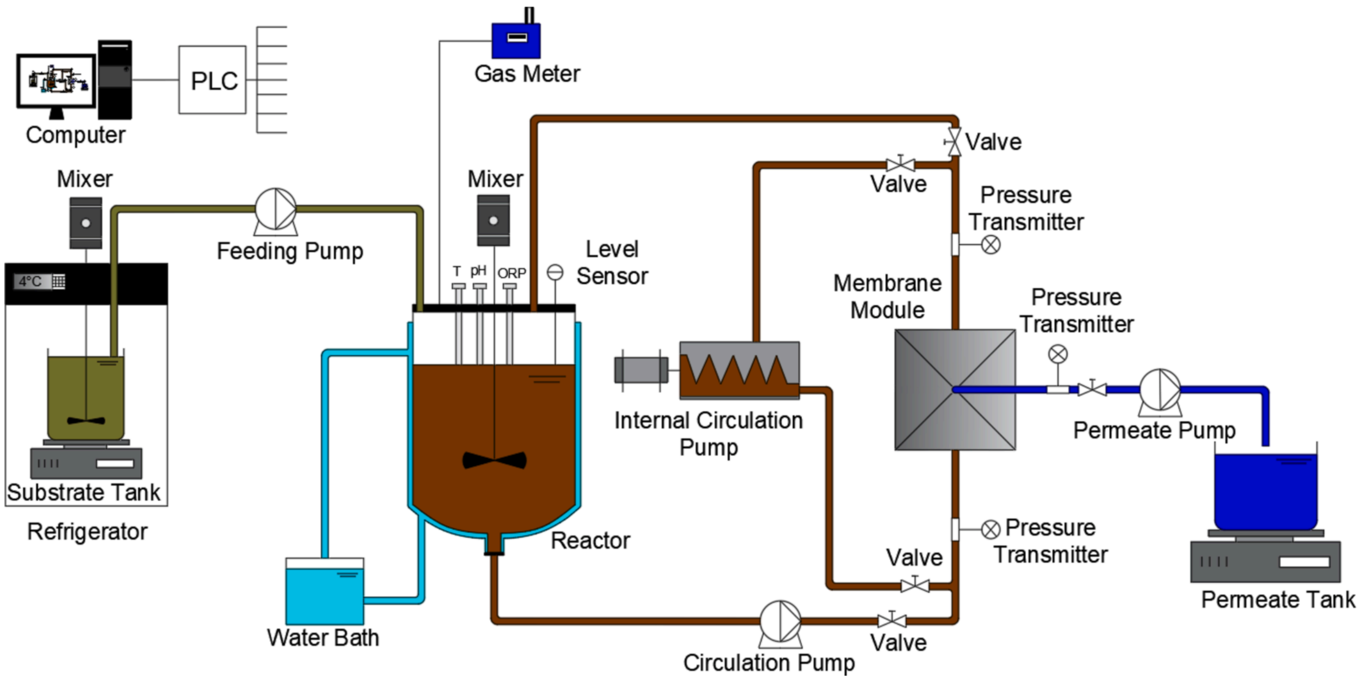

Fig. 1. Experimental set-up of the lab-scale AnMBR.

that was obtained from the sensors.

2.2. *Experimental procedure*

The AnMBR was operated at mesophilic (35 °C) and thermophilic (55 °C) conditions for 97 days in two separate operational periods. For the thermophilic AnMBR, the temperature of the inoculum was raised to 55 °C in one step. The HRT and SRT were maintained at 3.5 and 25 days, respectively. The organic loading rate (OLR) was kept at around 2 kg COD/(m^3·d) throughout the study. The membrane flux was kept stable at around 5 L/(m^2·h). The membrane was operated in cycles, including a 190-second filtration phase and 35-second backwash phase. The permeate was used to backwash the membrane at the same filtration flux. Biogas production was used as an indication of stable conditions when the daily fluctuation in biogas production for at least 10 days was less than 10 %. The reported average values in this study were determined based on the values during stable conditions.

2.3. *Inoculum and substrate characteristics*

The inoculum was obtained from a lab-scale mesophilic AnMBR system which was fed with A-sludge (Abdelrahman et al., 2022). The same inoculum was used for mesophilic and thermophilic AnMBRs. The average ratio between volatile suspended solids (VSS) and total suspended solids (TSS) concentrations was around 0.49. The AnMBR system was fed with sludge obtained from a pilot-scale A-stage (A-sludge) operated at HRT of 75 min, SRT of 0.5 d and DO concentration of 0.5 mg/L. The sludge was screened through a 2 mm screen to discard large particles and then stored at 4 °C. Table 1 illustrates the average values of inoculum and A-sludge characteristics during AnMBR operation at mesophilic and thermophilic conditions.

2.4. *Experimental analyses*

TS, VS, TSS, VSS, COD, sCOD, pH, TN, NH_4^+-N, coliforms, and alkalinity were measured according to Standard Methods (APHA, 2017). For sCOD measurements, the samples were filtered through a 0.45 μm filter before analysis. The d_{50} of substrates and anaerobic sludge were measured by a Mastersizer 2000 (Malvern Instruments, Hydro 2000 MU, UK). CST analyzer (Triton Electronics, Type 304 M, UK) was used for CST measurements. Soluble microbial products (SMP) were conducted for anaerobic sludge and permeate samples, which were filtered through

Table 1
Characteristics of the inoculum and substrate, A-sludge, during mesophilic and thermophilic operation.

Parameter	Unit	Inoculum[a]	Substrate at Mesophilic Condition	Substrate at Thermophilic Condition	No. of Samples
TSS	g/L	22.6 ± 1.1	7.1 ± 0.2	7.1 ± 0.1	33
VSS	g/L	11.0 ± 0.3	4.4 ± 0.2	4.2 ± 0.1	33
COD	g/L	16.0 ± 1.8	7.2 ± 0.2	7.9 ± 0.2	33
Soluble COD (sCOD)	g/L	0.9 ± 0.2	1.8 ± 0.1	2.1 ± 0.1	33
pH	–	7.5 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	33
Total nitrogen (TN)	mg/L	1030 ± 139	533 ± 15	549 ± 14	22
Total phosphorus (TP)	mg/L	199 ± 12	117 ± 4	114 ± 3	22
Dissolved phosphorus	mg/L	–	25 ± 1	24 ± 1	22
Ammonium-Nitrogen (NH_4^+-N)	mg/L	590 ± 3	208 ± 6	262 ± 10	22
Total Coliforms (TC)	MPN/gTS	–	$1.3x10^5 \pm 4.7x10^3$	$8.1x10^4 \pm 1.8x10^4$	11
Median particle size (d_{50})	μm	10.4 ± 0.4	52.4 ± 2.2	56.6 ± 3.2	11
CST	sec	179 ± 17	131 ± 4	97 ± 5	11
Alkalinity	mg $CaCO_3$/L	5175 ± 119	–	–	–
Specific methanogenic activity at 35 °C	g CH_4/(g VS·d)	0.094 ± 0.012	–	–	–

[a] Three samples were analyzed.

a 0.45 μm filter. Bound extracellular polymeric substances (EPS) including loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), were measured by the heat extraction method explained by Kinyua et al. (2017). The carbohydrate and protein fractions in SMP and bound EPS were measured by the phenol–sulfuric acid method (Dubois et al., 1956) and modified Lowry method (Frølund et al., 1995), respectively. Bovine serum albumin and D-glucose were used as standards for protein and carbohydrates assays. A gas chromatography (GC) equipped with a flame ionization detector (FID) (Agilent 7890 A, USA) was used to measure methane content in the biogas. Volatile fatty acids (VFAs) measurements were performed by using GC-FID (Shimadzu, Japan). The measured VFAs included acetate, propionate, butyrate, valerate, caproate and heptanoate. At the end of the operation, the specific methanogenic activity was measured for anaerobic sludge under mesophilic and thermophilic conditions as described in the study of Abdelrahman et al. (2022). The surface of a virgin membrane and the cake layers were imaged by using a scanning electron microscope (SEM) (Thermo Fisher Scientific Inc., FEI Quanta FEG 250 ESEM, UK). The membrane samples were coated by a layer of Palladium and Gold (Pd–Au) with a thickness of around 3–4 nm by using a vacuum evaporator (Quorum SC7620, UK) to increase the conductivity for SEM measurements. A Fourier transform infrared spectroscopy (FTIR) (Perkin-Elmer Inc., Spectrum 100 spectrometer, USA) was used to define the organic materials on the surface of the membranes.

2.5. Mass balance

The COD mass balance over the AnMBR expresses that the COD present in the influent leaves the reactor in the form of biogas, with the waste sludge or as dissolved COD or dissolved methane in the permeate (Equation (1):

$$Q_{in} \cdot COD_{in} = \left(\frac{Q_{Biogas} \cdot x_{CH4}}{0.35}\right) + (Q_{was} \cdot COD_{was}) + (Q_{Per} \cdot COD_{Per}) + (Q_{Per} \cdot COD_{CH4,\,diss}) \quad (1)$$

in which Q_{in} (L/d) is influent flow rate, COD_{in} (g/L) is COD concentration in influent, Q_{Biogas} (L/d) is the biogas flow rate, x_{CH4} (−) is the volume fraction of methane in biogas, 0.35 (L/g COD) is theoretical methane yield of COD, Q_{was} (L/d) is the waste sludge flow rate (L/d), COD_{was} (g/L) is COD concentration in waste sludge, Q_{Per} (L/d) is permeate flow rate, COD_{Per} (g/L) is COD concentration in permeate, $COD_{CH4,diss}$ (g/L) denotes the COD concentration of dissolved methane in permeate, which was estimated assuming equilibrium between the gas phase and the liquid phase as described in the study of Vergote et al. (2019) (Equation (2)):

$$COD_{CH4,\,diss} = 64 \cdot \frac{0.0014}{100} \cdot \exp\left(-\frac{14240}{R_h} \cdot \left(\frac{1}{298.15} - \frac{1}{T}\right)\right) \cdot p_{CH4} \quad (2)$$

in which 64 is COD content in methane (g COD/mol), 0.0014 is the Henry's law constant for methane in water at 25 °C (mole/(L·bar)), 100 is to convert bar to kPa, 14,240 is the enthalpy change associated with the dissolution of 1 mol of the methane (J/mol), R_h is ideal gas constant (8.3145 $Pa \cdot m^3/(mol \cdot K)$), T is the temperature (K), P_{CH4} is the partial pressure of methane in the headspace of AnMBR (kPa).

2.6. Mathematical modeling

2.6.1. Biological treatment model

The treatment performance and biogas production in the AnMBR under mesophilic and thermophilic conditions were simulated using ADM1. The matrix of the state variables in the ADM1 model and a list of values of all parameters (see supplementary materials) were adopted from Batstone et al. (2002). The mass balances were adjusted to reflect the uncoupling between SRT and HRT in AnMBR systems: permeate is represented by only soluble components, and waste sludge characteristics are the same as sludge inside the reactor. A factor "σ" was used to represent membrane retention efficiency for sCOD as shown in Equation (3) and (4):

$$\frac{dS_{liq,i}}{dt} = \frac{1}{V_{liq}}\left\{S_{in,i} \cdot Q_{in} - S_{liq,i} \cdot Q_{was} - S_{liq,i} \cdot Q_{per} \cdot (1-\sigma)\right\} + \sum_{j=1-19} \rho_j v_{i,j} \quad (3)$$

$$\frac{dX_{liq,i}}{dt} = \frac{1}{V_{liq}}\left\{X_{in,i} \cdot Q_{in} - X_{liq,i} \cdot Q_{was}\right\} + \sum_{j=1-19} \rho_j v_{i,j} \quad (4)$$

where S is the soluble state variables, Q is flow rate, V is the digester volume, σ is the membrane removal efficiency, X is the solids state variables, *t* is time, the term $\sum_{j=1-19}\rho_j v_{i,j}$ is the sum of the kinetic rates for process *j* multiplied by $\nu_{i,j}$, the subscript *liq* refers to liquid phase (inside the AnMBR), *i* means the component number defined in ADM1, *in* is the influent, *was* is the waste sludge, *per* is the permeate.

The influent sludge characteristics need to be identified. The COD in sludge consists mainly of inerts, proteins, carbohydrates and lipids. In this study, the COD in A-sludge was distributed as in the case of primary sludge. In the literature, it is reported that inert COD can be around 30–50 % of the total COD of municipal sewage sludge (Siegrist et al., 2002; Mendes et al., 2015). Then, the rest of COD is distributed among proteins, lipids and carbohydrates (Gernaey et al. 2014). The proteins COD concentration in the sludge is calculated as: (Total Kjeldahl nitrogen – NH_4^+-N) x 6.25 x 1.47 (Abdelrahman et al., 2023). Later, the leftover of COD (100 %) in primary sludge is distributed between lipids (~70 %) and carbohydrates (~30 %) (Gernaey et al. 2014). Therefore, in our present research, the measured COD of influent sludge was divided into 40 % particulate inert, 3 % soluble inert, 31 % proteins, 17 % lipids and 9 % carbohydrates. The biokinetic model was implemented in the AQUASIM v2.1 software. The results of biogas production and composition, COD removal efficiency, and pH, were considered validated when the relative absolute error was less than 10 %.

2.6.2. Membrane fouling

The membrane fouling behavior under mesophilic and thermophilic conditions was identified by simulating the TMP according to the method described by Charfi et al. (2015). The TMP (mbar) is a function of a set of membrane filtration resistances as shown in Equation (5):

$$TMP = \frac{\mu \cdot J \cdot (R_0 + R_s + R_c)}{3600 \cdot 100 \cdot 1000} \quad (5)$$

where μ is the dynamic viscosity (kg/(m·s)), J is the permeate flux (L/(m^2·h)), R_0 is the membrane intrinsic resistance (m^{-1}), R_s is thin fouling layer resistance (m^{-1}), R_c is the cake resistance (m^{-1}), 3600 refers to s/h, 1000 refers to L/m^3, 100 corrects Pa to mbar. The values of the observed TMP were used to calibrate the model and to calculate these different resistances. The parameter values used in the model are listed in the supplementary materials (see supplementary materials).

2.7. Energy balance

The net energy recovery in the AnMBR system (E_N, in J/g COD) was calculated as the difference between energy consumption and recovery (Equation (6). Energy recovery included energy produced from biogas (methane) (E_G) and heat recovery (E_{HR}) from waste sludge and permeate to heat the influent via a heat exchanger, which was assumed to be present, as in the study of Cheng et al. (2021). Energy consumption included energy for influent heating (E_{IH}) and heat loss (E_{HL}), reactor mixing (E_M), sludge pumping (E_P) and permeate extraction (E_{Per}).

$$E_N = E_G + E_{HR} - E_{IH} - E_{HL} - E_M - E_P - E_{Per} \quad (6)$$

Energy production was calculated based on energy (electricity and heat) recovery from methane (biogas) production (E_G, in J/d) using the combined heat and power unit based on Equation (4):

$$E_G = Q_m \cdot CV_m \cdot E \cdot 3600/1000 \tag{7}$$

Where Q_m is average methane daily production (m^3/d), CV_m is calorific value of methane (kWh/m^3), E is electricity and heat conversion efficiency (−), 3600 corrects for h to s, and 1000 corrects kW to W. The heat recovery (E_{HR}, in J/d) from the heat exchanger was calculated based on Equation (5). The heat required for influent heating (E_{IH}, in J/d) and heat loss (E_{HL}, in J/d) were calculated based on Equations (6) and (7), respectively:

$$E_{HR} = Q_{Influent} \cdot \left(T_{AD} - T_{Influent}\right) \cdot \rho \cdot C_p \cdot \Phi \tag{8}$$

$$E_{IH} = Q_{Influent} \cdot \left(T_{AD} - T_{Influent}\right) \cdot \rho \cdot C_p \tag{9}$$

$$E_{HL} = A \cdot \left(T_{AD} - T_{sur}\right) \cdot U \cdot 86,400 \tag{10}$$

Where, T_{AD} is the operational temperature of the AnMBR (°C), $T_{influent}$ is the temperature of the influent sludge (°C), ρ is the sludge density (kg/m^3) and C_p its specific heat capacity (J/(kg·°C)), Φ is heat recovery efficiency by heat exchanger (−), A is AnMBR surface area (m^2), T_{sur} is the surrounding temperature (°C), U is heat coefficient of heat transfer from walls ($W/(m^2 \cdot °C)$), and 86,400 corrects for d to s. The energy consumed for mixing the reactor content (E_M, in J/d) was calculated based on Equation (8):

$$E_M = V \cdot \omega \tag{11}$$

Where, V is the volume of the AnMBR (L), ω is specific power of the stirrer (J/(L·d)). Energy consumed for sludge pumping (E_P, in J/d) for feeding, sludge circulation and internal circulation was determined based on Equation (9):

$$E_P = \frac{Q_{sludge} \cdot H \cdot \rho \cdot g}{e} \tag{12}$$

Where, Q_{sludge} is sludge flow rate ($m^3 \cdot d^{-1}$), H is the head pressure (m), g is the gravitational acceleration (m/s^2), e is the pump efficiency (−). Energy consumption by permeate pump (E_{Per}, in J/d) was calculated as shown in Equation (10):

$$E_{Per} = \frac{Q_{Permeate} \cdot TMP \cdot 100}{e} \tag{13}$$

Where, TMP is the transmembrane pressure (mbar), and 100 corrects Pa to mbar. All energy values (E_i, in J/d) were normalized based on the amount of COD in the influent as shown in Equation (11):

$$E_j = \frac{E_i}{Q_{Influent} \cdot COD_{Influent}} \tag{14}$$

Where E_j is the energy value in J/g COD.

Two scenarios were developed to calculate the net energy recovery in the AnMBR. For the first scenario, the energy consumption and recovery were calculated at influent COD concentrations of around 7 g/L based on the experimental results and ADM1. In practice, the activated sludge is thickened, in which COD concentration reaches around 25 g/L (Tchobanoglous et al., 2014). Therefore, to evaluate the energy balance, another scenario was created by considering the influent flow rate the same and the COD concentration at 25 g/L. In this scenario, ADM1 was used to estimate the biogas production by using the same kinetic parameters used in the model for the first scenario. Membrane fouling model was used for TMP estimation to calculate energy for filtration.

3. Results and Discussion

3.1. Biogas production and process stability

The experimentally observed average biogas production was 0.69 ± 0.04 L-biogas/L-reactor/d and 0.84 ± 0.03 L-biogas/L-reactor/d in mesophilic and thermophilic AnMBRs, respectively (Fig. 2a and b). This implies a 23 % higher biogas production during digestion of A-sludge in thermophilic AnMBR compared to a mesophilic one. A higher specific methane yield was obtained in thermophilic AnMBR (0.23 ± 0.01 L CH_4/g COD_{fed}), compared to mesophilic AnMBR (0.20 ± 0.01 L CH_4/g COD_{fed}). Enhanced hydrolysis and faster biochemical reactions are reported to be the main reason for higher methane yield under thermophilic conditions (Gebreeyessus, 2020). The high methane yield of A-sludge was reported to be similar to that of primary sludge, however, the lag phase of A-sludge digestion (1 day) was shorter compared to primary sludge (2.2 days) (Abdelrahman et al., 2023). The experimentally observed biogas production and composition were well-fitted with the ADM1 model with a relative absolute error of less than 6 %. Biochemical reaction rates were higher in the thermophilic AnMBR, in particular the disintegration rate of particulates and degradation rates of amino acid, sugar, long chain fatty acid and VFAs (see supplementary materials).

Specific methanogenic activity is another parameter to evaluate microbial degradation rates of organics since methanogenesis is a rate-limiting step in the anaerobic digestion process. Like biogas production, the specific methanogenic activity of thermophilic sludge, i.e., 0.19 ± 0.00 g CH_4/(g VS·d), was higher than that of mesophilic sludge, which was 0.11 ± 0.00 g CH_4/(g VS·d). Kim et al. (2017) reported an increase in the activity of methanogens after elevating the temperature of a methanogenic tank of a two-temperature-phased AnMBR from 35 °C to 55 °C.

The stability of digesters can be followed by alkalinity and VFA concentrations in the anaerobic sludge (Chen et al., 2019). Alkalinity was similar in both AnMBRs in which the average alkalinity was 5211 ± 358 and 5189 ± 261 mg $CaCO_3$/L in the mesophilic and thermophilic AnMBRs, respectively. Cook et al. (2017) reported that an alkalinity of more than 2000 mg $CaCO_3$/L indicates a stable operation for digesters. In this study, a higher VFA concentration was observed in the thermophilic AnMBR compared to the mesophilic one (Fig. 2c and d), with average VFA concentrations in the mesophilic and thermophilic AnMBRs of 379 ± 80 and 559 ± 49 mg/L, respectively. The higher VFA concentration in the thermophilic reactor was reported to be a result of faster biochemical reactions under thermophilic conditions (Nges and Liu, 2010), which was confirmed by ADM1 (see supplementary materials). In addition, the composition of VFAs was different between the two AnMBRs over the operational period. Acetate, butyrate, valerate and heptanoate were the dominant VFAs at the beginning of the mesophilic operation. However, after the start-up, the composition of VFAs changed in which acetate, propionate and butyrate were the dominant VFAs, representing around 61, 16 and 14 % of total VFAs on average, respectively. While all VFAs were present in the anaerobic sludge at the beginning of the thermophilic operation. Only heptanoate was not present in the anaerobic sludge after the start-up. During the thermophilic operation, acetate, propionate, butyrate, valerate and caproate constituted around 60, 11, 7, 10 and 12 % of the total VFAs on average, respectively. VFA to alkalinity ratios in the mesophilic and thermophilic AnMBRs were around 0.07 and 0.11, respectively. These values are lower than the proposed inhibition level (VFA/alkalinity = 0.3), in which there would be an accumulation of VFA (Liu et al., 2012). Apparently, both AnMBRs were operated under stable conditions. Low concentrations of VFAs (< 100 mg/L), mainly acetate, were found in the permeate of the mesophilic AnMBR. In the permeate of the thermophilic AnMBR, the VFA concentration reached more than 600 mg/L on the 13th day (Start-up period), then decreased quickly during operation (Fig. 2e and f). However, no VFAs were detected in both permeates after 61 days till the end of the operation.

3.2. Treatment performance

The treatment performances of the mesophilic and thermophilic AnMBRs were comparable (Table 2). High COD removal efficiencies

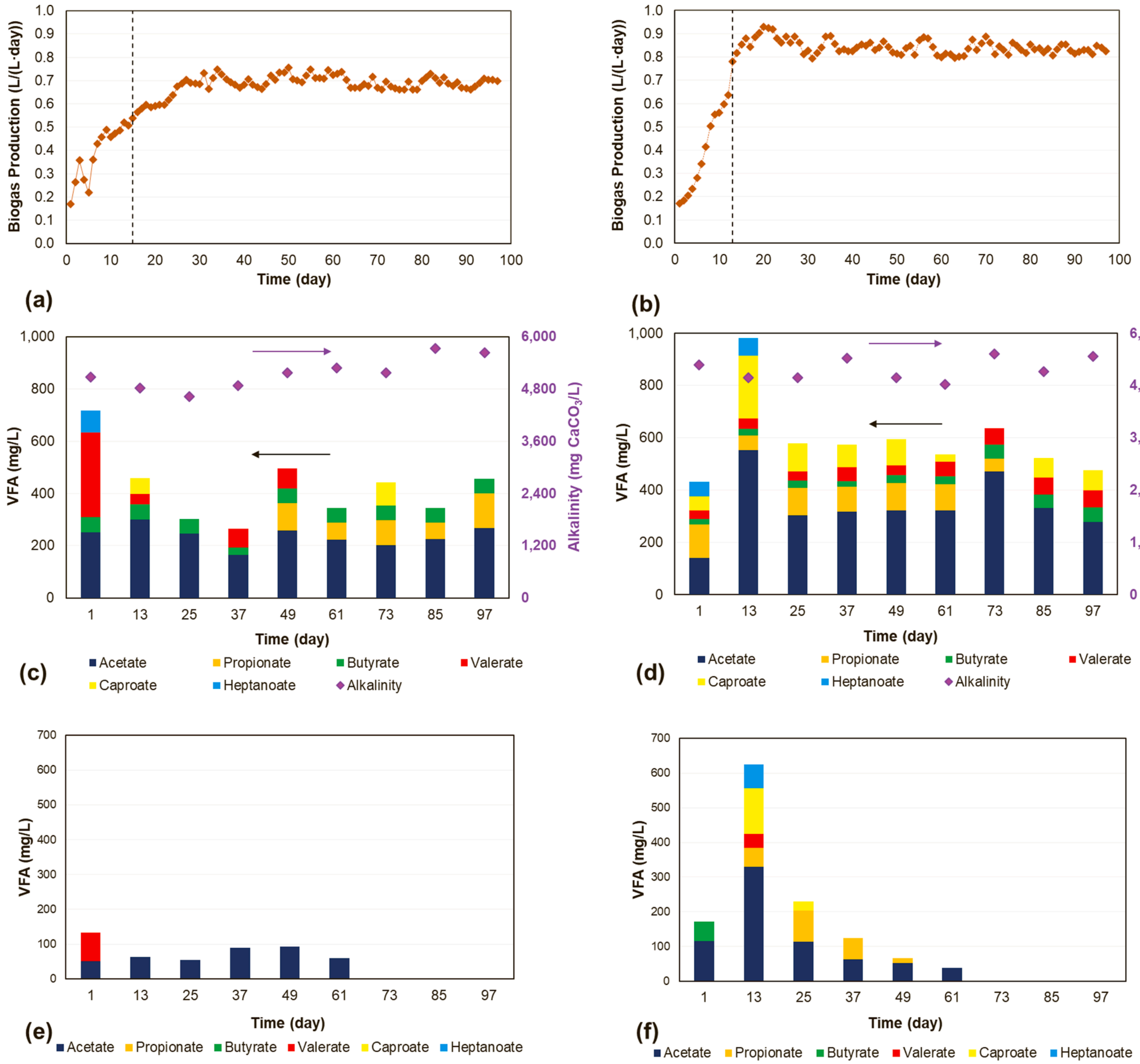

Fig. 2. Biogas production: (a) mesophilic AnMBR, (b) thermophilic AnMBR; VFA concentrations inside: (c) mesophilic AnMBR, (d) thermophilic AnMBR; VFA concentrations in the permeates of: (e) mesophilic AnMBR, (f) thermophilic AnMBR.

Table 2
Treatment performance of the AnMBR.

Parameter	Mesophilic AnMBR				Thermophilic AnMBR				No. of Samples
	Reactor (g/L)	Influent (g/L)	Permeate (g/L)	Removal Efficiency (%)	Reactor (g/L)	Influent (g/L)	Permeate (g/L)	Removal Efficiency (%)	
COD	17.8 ± 0.8	7.2 ± 0.2	0.2 ± 0.0	97.1 ± 0.2	17.1 ± 0.3	7.9 ± 0.2	0.3 ± 0.1	95.8 ± 1.9	33
sCOD	0.9 ± 0.1	1.8 ± 0.1	0.2 ± 0.0	89.5 ± 0.8	1.2 ± 0.1	2.1 ± 0.1	0.3 ± 0.1	85.7 ± 0.8	33
TN	1107 ± 44[a]	533 ± 15[a]	430 ± 12[a]	19.3 ± 1.0	1052 ± 58[a]	549 ± 14[a]	448 ± 21[a]	18.2 ± 3.4	22
NH_4^+-N	554 ± 20[a]	208 ± 6[a]	337 ± 11[a]	–	570 ± 11[a]	262 ± 10[a]	379 ± 15[a]	–	22
TSS	24.7 ± 0.9	7.1 ± 0.2	ND [c]	> 99.9	22.5 ± 1.0	7.1 ± 0.1	ND [c]	> 99.9	33
VSS	12.6 ± 0.4	4.4 ± 0.2	ND [c]	> 99.9	11.4 ± 0.5	4.2 ± 0.1	ND [c]	> 99.9	33
TC	–	$1.3x10^5$ ± $4.7x10^3$ [b]	ND [c]	100	–	$8.1x10^4$ ± $1.8x10^4$ [b]	ND [c]	100	11
pH	7.3 ± 0.1 [d]	6.7 ± 0.1[d]	7.6 ± 0.2 [d]	–	7.1 ± 0.1 [d]	6.8 ± 0.1 [d]	7.7 ± 0.1 [d]	–	33

[a] mg/L, [b]MPN/g TS, [c]Not detected, [d]unitless.

were achieved by the AnMBR under both mesophilic and thermophilic conditions, reaching around 97 and 96 %, respectively. The COD removal efficiency of both AnMBRs were well-fitted by the ADM1, with a relative error of less than 4 %. The average COD in the permeate of the thermophilic AnMBR was slightly higher than that of the mesophilic AnMBR. This higher value was related to higher VFA concentration in the permeate of the thermophilic AnMBR. A-sludge contains a high amount of proteins, thus, the TN concentration is relatively high compared with primary sludge. Therefore, more soluble proteins and NH_4^+-N (a by-product from protein hydrolysis) can be expected in the reactor (Abdelrahman et al., 2023). Low TN removal efficiency was observed in both AnMBRs, which was expected since the soluble proteins and NH_4^+-N could pass through membranes. The rinsing of accumulating NH_4^+-N from the AnMBR can help to provide stable conditions for the anaerobic process, preventing inhibition, as observed in the study of Kanai et al. (2010). The NH_4^+-N concentrations in both AnMBRs were out of the inhibition level (4000 mg/L) (Procházka et al., 2012).

Biomass properties including EPS and SMP can be affected by shifts in temperature (Sierra et al., 2018). The thermophilic conditions might have induced the release of SMP, including proteins and carbohydrates, which were more than double the amount under mesophilic conditions (Table 3). In addition, lower LB- and TB-EPS concentrations were measured in the thermophilic AnMBR, in which bound EPS may be hydrolyzed and converted to soluble form (SMP). Similar findings were detected in the study of Manzoor et al. (2022), in which a trend of SMP increase and decrease in bound EPS was found by increasing the temperature of an AnMBR (35, 40, 45 and 50 °C) treating textile wastewater. Comte et al. (2006) reported that an increase in SMP may be related to hydrolysis of organics in the reactor, hydrolysis of bound EPS, and/or cell lysis. Higher protein concentrations in all EPS than carbohydrates can be correlated with the presence of exoenzymes in the sludge flocs (Frølund et al., 1995). Permeate quality can be affected by SMP concentration since these soluble products can partly pass through the membranes. Likely therefore, higher SMP concentrations were found in the permeate of the thermophilic reactor compared to the mesophilic reactor. This finding agreed with the higher COD concentrations in the permeate of the thermophilic AnMBR.

A COD mass balance was conducted to have a better understanding of the fate of the organics in the mesophilic and thermophilic AnMBRs (Fig. 3). By using AnMBR for sludge digestion, around 57 % of the organics (COD) in the influent sludge could be recovered into methane gas at mesophilic conditions, while 65 % of COD could be converted into methane gas at thermophilic conditions. Only 2.5 and 3.5 % of influent sludge COD was found in the permeate of mesophilic and thermophilic AnMBRs, respectively, showing high removal efficiencies by both AnMBRs. Waste sludge contained around 4 % more influent COD under mesophilic conditions, which indicates higher organics degradation under thermophilic conditions. The concentration of dissolved methane was even lower under thermophilic (0.4 %) than mesophilic conditions (0.6 %) reflecting a decreased solubility of gaseous compounds at higher temperatures. In practice, a membrane contactor can be used to recover the dissolved methane from the permeate (Lim et al., 2019).

Table 3
SMP and EPS compositions in the AnMBR and permeate.

SMP and EPS compositions	Unit	Mesophilic AnMBR	Thermophilic AnMBR
SMP in reactor			
Protein	mg/g VSS	10.8 ± 0.8	24.1 ± 5.7
Carbohydrates	mg/g VSS	4.2 ± 0.6	8.9 ± 1.3
Protein/Carbohydrates	–	2.6 ± 0.5	2.8 ± 0.8
LB-EPS in reactor			
Protein	mg/g VSS	23.2 ± 3.3	20.9 ± 17.6
Carbohydrates	mg/g VSS	3.5 ± 1.1	3.8 ± 1.9
Protein/Carbohydrates	–	7.3 ± 2.2	5.4 ± 2.4
TB-EPS in reactor			
Protein	mg/g VSS	38.9 ± 3.6	30.3 ± 8.0
Carbohydrates	mg/g VSS	7.3 ± 1.6	5.3 ± 1.0
Protein/Carbohydrates	–	5.6 ± 1.2	5.8 ± 1.4
Total bound EPS in reactor			
Protein	mg/g VSS	62.1 ± 3.2	51.1 ± 2.8
Carbohydrates	mg/g VSS	10.7 ± 2.5	9.1 ± 1.8
Protein/Carbohydrates	–	6.1 ± 1.4	5.6 ± 1.6
SMP in permeate			
Protein	mg/L	127.4 ± 16.9	149.3 ± 50.2
Carbohydrates	mg/L	11.2 ± 7.7	18.8 ± 4.6

* Six samples were analyzed throught the operation under each condition.

3.3. Transmembrane pressure and filtration performance

The membranes were operated at a flux of 5 L/(m^2·h) after increasing the flux gradually in the first few days. The membrane unit in the thermophilic AnMBR was operated at stable TMP for a longer period compared to its mesophilic counterpart, while TMP increased at a higher rate in the mesophilic one, especially after day 69 (Fig. 4). The average TMP values were 117 ± 45 and 98 ± 15 mbar for mesophilic and thermophilic AnMBRs, respectively. Higher TMP values in the mesophilic AnMBR were attributed to the higher bound EPS values in the mesophilic sludge. Wang et al. (2009) reported that membrane fouling had a significant positive correlation with bound EPS, especially LB-EPS, which had an inverse correlation with temperature. Hydrodynamics characteristics of the anaerobic sludge may affect membrane fouling, in which viscosity is dependent on temperature. Meabe et al. (2013) reported that lower viscosity of sludge at higher temperatures allowed to operate a thermophilic AnMBR with higher membrane permeability and lower TMP for a longer period of time compared to a mesophilic one. However, the results of this study were in contradiction with the results of the study of Jeison and Van Lier (2008), in which membrane filtration resistance of an AnMBR treating wastewater was higher at thermophilic conditions. It was reported that the particles in the thermophilic AnMBR were smaller due to disintegration, and they deposited on the membrane surface, causing an increase in TMP. The operational TMP was fitted with the TMP from membrane fouling model ($R^2 > 0.84$) to understand the contribution of each filtration resistance in membrane fouling (Fig. 4). The first thin layer resistance was higher in the thermophilic AnMBR (2.3 x 10^7 m^{-1}) compared to the mesophilic one (0.1 x 10^7 m^{-1}) due to higher sCOD concentrations in the thermophilic AnMBR. While cake layer resistance was lower in the thermophilic AnMBR (2.9 x 10^{12} m^{-1}) compared to the mesophilic one (4.3 x 10^{12} m^{-1}), which was attributed to higher particulate COD in the mesophilic reactor. More details about filtration resistance are provided in the supplementary materials (see supplementary materials).

3.4. Morphological analyses

The surfaces of the virgin membrane and cake layers under both conditions were imaged by ESEM at the end of the operation (see supplementary materials). Crystal-like materials can be observed on the surface of both cake layers. Struvite ($MgNH_4PO_4·6H_2O$) is a known inorganic foulants that can accumulate on the surface of the membrane and inside membrane pores (Yoon et al., 1999). A denser cake layer was accumulated on the surface of the membrane under mesophilic conditions. This dense layer may be related to higher EPS concentration of anaerobic sludge under mesophilic conditions. Niu et al. (2020) indicated that EPS and inorganic particles together could form a thick cake layer, which caused a high membrane filtration resistance. Meabe et al. (2013) stated that fouling was related to the viscosity of sludge in mesophilic AnMBR, and to pore blocking by inorganic foulants in thermophilic AnMBR. Therefore, the denser cake layer and higher viscosity may cause the faster increase in TMP in the mesophilic AnMBR.

The chemical structure of organic foulants on the membranes was characterized by FTIR analysis (see supplementary materials). Similar peaks were observed on membrane surfaces of mesophilic and

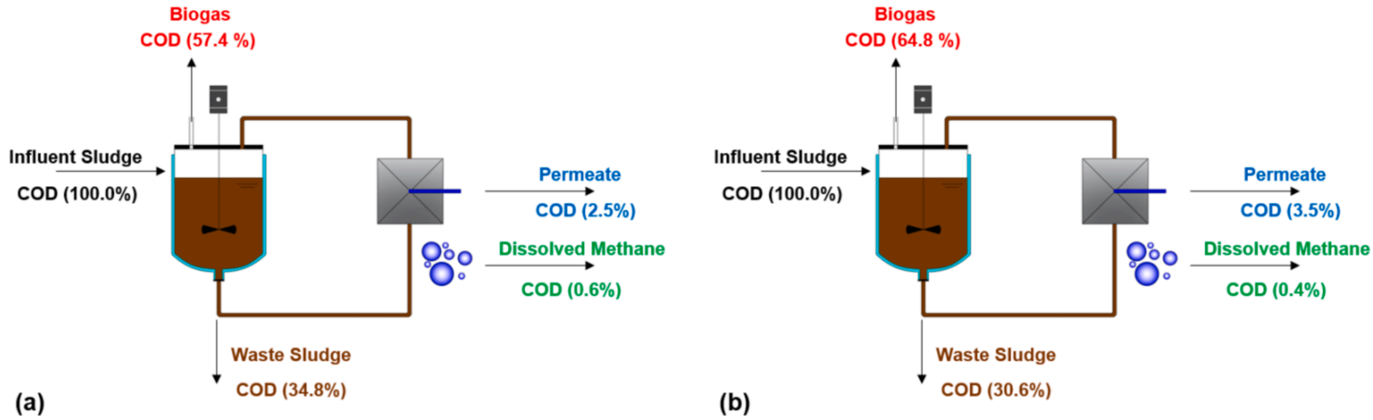

Fig. 3. COD mass balance: (a) mesophilic AnMBR; (b) thermophilic AnMBR.

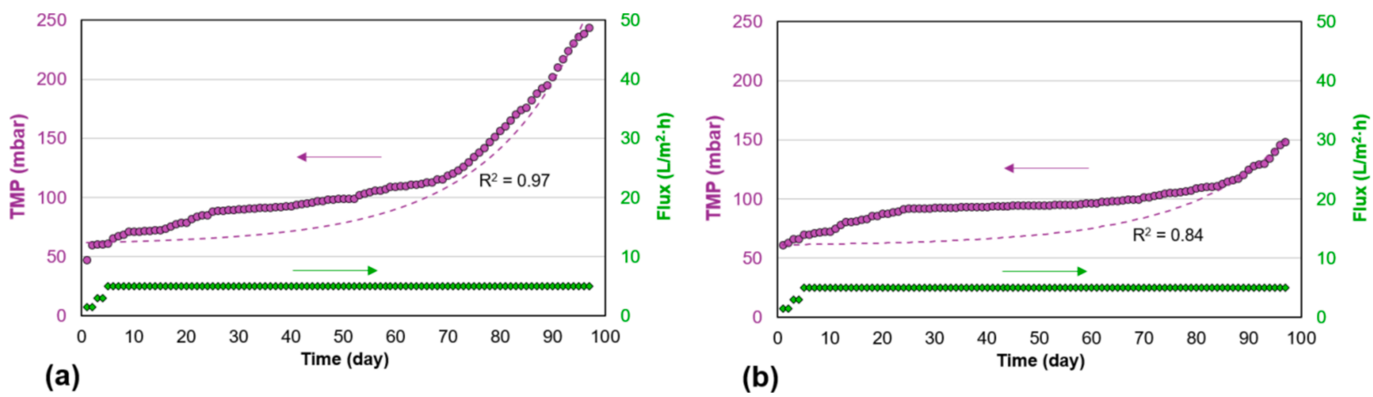

Fig. 4. TMP profiles: (a) mesophilic AnMBR, (b) thermophilic AnMBR. Dashed line represents the simulated TMP.

thermophilic AnMBRs. The two peaks observed at 3278 cm^{-1} in the spectrum indicated the structure of polysaccharides (O-H) (Ersahin et al., 2016). The peak at 2917 cm^{-1} corresponded to aliphatic C-H stretching (polysaccharides). The peaks at 1632 and 1535 cm^{-1} represented the existence of amides I (C = O and C-N) and amides II (N-H and C-N), respectively (Aslam et al., 2018, Balcıoğlu et al., 2021, Gao et al., 2016). Amides III (N-H and C-N) were represented by the peaks at 1230 and 1452 cm^{-1} (Ersahin et al., 2016). The peaks observed at 1047 cm^{-1} exhibited the polysaccharides or polysaccharides-like substances (Croué et al., 2003). Peaks in the fingerprint region (< 1000 cm^{-1}) could

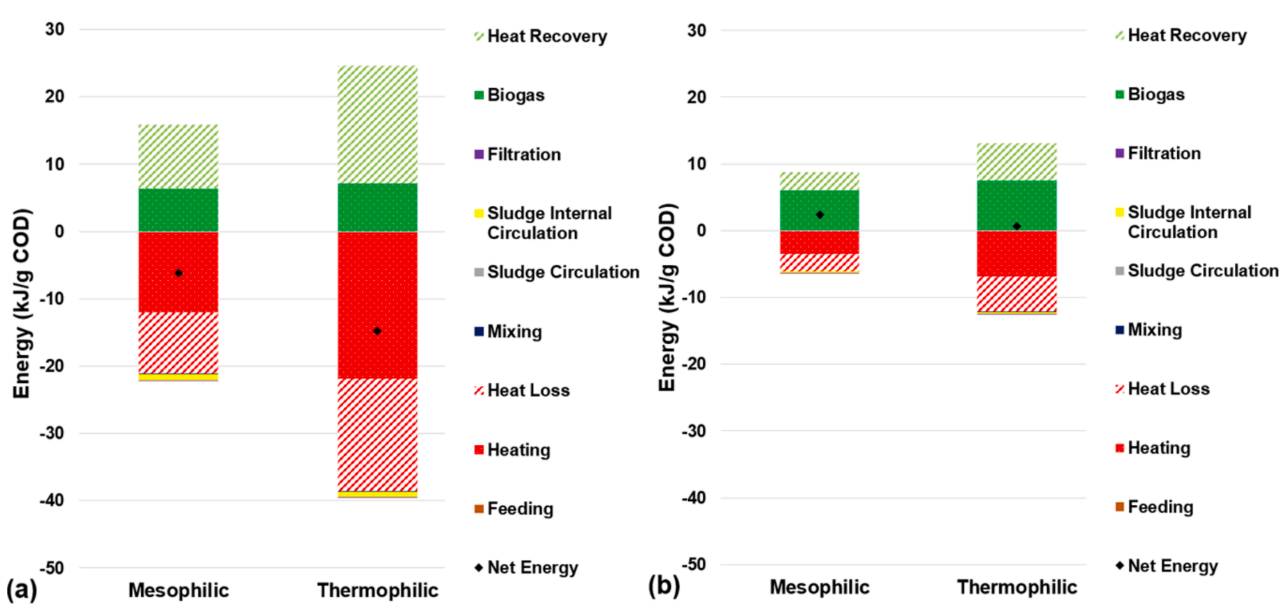

Fig. 5. Energy consumption (negative values) and recovery (positive values) in mesophilic and thermophilic AnMBRs expressed per unit influent COD concentration: (a) ~ 7 g/L, (b) 25 g/L (ADM1 model).

represent different functional groups, phosphate and sulfate groups, originated from nucleic acids (Gao et al., 2011). Based on the FTIR spectra results, protein- and polysaccharide-like substances (EPS and SMP) were the main organic substances accumulated on the surface of the membranes in both AnMBRs.

3.5. Energy balances

The biogas production at thermophilic conditions was 23 % higher than at mesophilic conditions, i.e., 7.1 kJ/ gCOD versus 6.3 kJ/ gCOD, respectively. Moreover, substantially more (83 %) heat could be recovered from the thermophilic sludge and permeate compared to the mesophilic ones, i.e., 9.6 kJ/g COD versus 17.6 kJ/g COD, respectively. However, thermophilic operation also entails higher energy demands for influent sludge heating, i.e., 21.9 kJ/ gCOD versus 12.0 kJ/ gCOD for mesophilic operation, and higher heat losses, i.e., 16.6 kJ/ gCOD versus 9.1 kJ/ gCOD for mesophilic operation. The higher energy demand resulted in a higher total energy consumption for the thermophilic AnMBRs (39.5 kJ/g COD) compared to the mesophilic one (22.1 kJ/ gCOD, see Fig. 5a). Only a small fraction of energy consumption was due to sludge pumping, mixing and permeate extraction (< 1.0 kJ/g COD) for both AnMBRs. A similar observation was reported in the study of Dagnew et al. (2013), in which energy consumption was mainly for influent sludge heating and heat loss of a mesophilic AnMBR treating waste activated sludge, accounting more than 96 % of the total energy consumption. The high energy consumption in the thermophilic AnMBR resulted in a lower net energy than the mesophilic AnMBR. The net energy balance of mesophilic and thermophilic AnMBR was negative, reaching −6.2 and −14.2 kJ/g COD, respectively.

The negative net energy balances obtained were attributed to the low COD concentration of the influent, resulting in a large energy demand for influent heating. In practice, the diluted activated sludge stream is thickened before anaerobic digestion, up to typical concentrations of 25 g/L (Tchobanoglous et al., 2014). The treatment of a more concentrated stream in the AnMBR has a large influence on the overall energy balance (Fig. 5b). Energy recovery from biogas production per g COD was similar to the treatment of sludge at lower COD concentration (7 g/L), reaching 6.0 kJ/g COD for mesophilic operation compared to 7.55 kJ/g COD for thermophilic one. However, the heat recovery per g COD was less in both AnMBRs, i.e., 2.76 and 5.52 kJ/g COD for mesophilic and thermophilic operations, respectively, since the flow rate was the same as in the case of influent COD of 7 g/L. The reduced flow resulted in less energy requirement for heating of influent sludge, i.e., 3.4 kJ/g COD for mesophilic operation versus 6.9 for the thermophilic one. Similarly, less heat loss was calculated, being 2.6 and 5.2 kJ/g COD for mesophilic and thermophilic operations, respectively. Overall, a net positive energy balance was assessed for both mesophilic and thermophilic conditions by introducing a sludge thickener prior to treatment, changing both AnMBRs from net energy consumers to net energy producers. However, three times more net energy could be produced under mesophilic conditions (2.41 kJ/g COD) than under thermophilic conditions (0.65 kJ/g COD), with the remaining heating requirements being the problem. Therefore, despite the higher biogas production under thermophilic conditions, operating AnMBR for sludge digestion at mesophilic conditions results in a higher net energy recovery.

4. Implications and recommendations

The conventional treatment of low-strength wastewater is energy-consuming, in which primary clarifier redirects only around 30–40 % of the organics from the wastewater into the sludge stream (primary sludge). The remained organics are sent to aeration tanks, in which around 30 % of the organics is converted into carbon dioxide, and 10 % of the organics leaves with the effluent, while the rest ends up in the waste activated sludge, which has low biodegradability (Guven et al., 2019). Therefore, adopting A-stage instead of the primary clarifier has the potential to maximize the organics harvesting for energy recovery from anaerobic digestion. Thus, the A-stage can be followed by an energy-efficient nutrient removal system such as partial nitritation anammox, which requires an influent with a very low carbon-to-nitrogen ratio (Abdelrahman et al., 2022). A-sludge digestion using AnMBR offers not only low footprint but also an opportunity for simultaneous nitrogen and phosphorous recovery from the permeate. However, based on the energy analysis in this study, thickening of A-sludge is important to achieve a positive net energy for anaerobic digestion.

In this study, the OLR was set the same for mesophilic and thermophilic AnMBR to understand the effect of the temperature on the treatment and filtration performance of the AnMBR digesting A-sludge. However, the advantage of the operation under thermophilic conditions is to increase the OLR or decrease the SRT, which was not considered in this study. Therefore, it is recommended to investigate the effect of increasing the OLR on the performance of AnMBR under both conditions. Besides, under thermophilic conditions, better sludge filtration performance is observed. For being conclusive in decision making, a detailed feasibility analysis is required that includes costs for sludge filtration, costs of membrane modules, and costs for chemical cleaning requirements at each condition.

5. Conclusions

Anaerobic digestion of A-sludge in lab-scale AnMBRs was compared for mesophilic and thermophilic conditions. Higher methane yield was observed in the thermophilic AnMBR (0.23 L CH_4/g COD_{fed}) compared to the mesophilic one (0.20 L CH_4/g COD_{fed}). The VFA concentrations were higher inside the thermophilic AnMBR and its permeate. The thermophilic AnMBR was operated at lower TMP, which was attributed to a relatively thin cake layer on the membrane surface. Operating the AnMBR under mesophilic conditions resulted in a more than three-fold higher net energy recovery than operating the AnMBR under thermophilic conditions due to the high energy required for heating.

CRediT authorship contribution statement

Amr Mustafa Abdelrahman: Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation, Conceptualization. **Saba Aghdam Tabar:** Writing – original draft, Investigation. **Busra Cicekalan:** Writing – original draft, Investigation. **Safak Basa:** Funding acquisition. **Gulin Ucas:** Funding acquisition. **Huseyin Guven:** Writing – review & editing, Project administration, Conceptualization. **Hale Ozgun:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Izzet Ozturk:** Writing – review & editing, Project administration. **Ismail Koyuncu:** Writing – review & editing, Resources. **Jules B. van Lier:** Writing – review & editing, Conceptualization. **Eveline I.P. Volcke:** . **Mustafa Evren Ersahin:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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.

Acknowledgements

This research was funded by Istanbul Water and Sewerage Administration (ISKI) with the project titled “Integration of High-rate Activated Sludge Process and Anaerobic Membrane Bioreactor Process for Energy Efficient Wastewater Treatment in Istanbul: Maximum Energy Recovery (MEGA2 Project)”. The authors express their gratitude for the PhD Fellowship award provided by the Turkish Academy of Sciences (TUBA) to Amr Mustafa Abdelrahman. They also gratefully acknowledge the support by the Istanbul Technical University, Turkey, Scientific Research Projects: Amr Mustafa Abdelrahman (Project No: MDK-2021-42990) and Saba Aghdam Tabar (Project No: MYL-2021-43105).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.131822.

Data availability

No data was used for the research described in the article.

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