Evaluation of a sponge assisted-granular anaerobic membrane bioreactor (SG-AnMBR) for municipal wastewater treatment

C. Chen\textsuperscript{a}, W.S. Guo\textsuperscript{a,}\textsuperscript{*}, H. H. Ngo\textsuperscript{a}, Y. Liu\textsuperscript{a}, B. Du\textsuperscript{b}, Q. Wei\textsuperscript{c}, D. Wei\textsuperscript{b}, D. D. Nguyen\textsuperscript{d}, S. W. Chang\textsuperscript{d}

\textsuperscript{a}Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, NSW 2007, Australia
\textsuperscript{b}School of Resources and Environment, University of Jinan, Jinan 250022, PR China
\textsuperscript{c}Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China
\textsuperscript{d}Department of Environmental Energy & Engineering, Kyonggi University, 442-760, Republic of Korea

* Corresponding author: Email: wguo@uts.edu.au, Tel: +61 (2) 95142739, Fax: +61(2) 95147803

Abstract

This study compared a conventional granular anaerobic membrane bioreactor (CG-AnMBR) with a sponge assisted-granular anaerobic membrane bioreactor (SG-AnMBR) in terms of treatment performance, granular sludge properties, membrane fouling behaviour and biogas production. The SG-AnMBR showed better organics and nutrient removal, and enhanced methane yield at 156.3 ± 5.8 mL CH\textsubscript{4} (STP)/g COD\textsubscript{removed}. Granular sludge from the SG-AnMBR had superior quality with better settleability, larger particle size, higher EPS content and more granule abundance. The SG-AnMBR also exhibited slower fouling development with 50.7% lower total filtration resistance than those of the CG-AnMBR. Sponge addition effectively affected the concentration and properties of microbial products (e.g. soluble microbial products (SMP) and extracellular polymeric substances (EPS)) in granular sludge, cake layer as well as settling zone mixed liquor, thus alleviating the fouling propensity. The liquid chromatography-organic carbon detection (LC-OCD) analysis suggested that sponge...
addition reduced the concentrations of biopolymers, low molecular weight neutrals and acids, and building blocks of the foulants. Compared with the SG-AnMBR, GC-MS analysis confirmed the accumulation of volatile fatty acids, particularly acetic acid in the CG-AnMBR. It is evident that the SG-AnMBR could be a promising solution for improving overall G-AnMBR performance and substantially mitigating membrane fouling.

**Keywords**: Anaerobic membrane bioreactor (AnMBR); Sponge; Granular sludge; Membrane fouling; Biogas production

1. Introduction

In the past decades, anaerobic membrane bioreactors (AnMBRs) have been found particularly attractive for wastewater treatment because it can not only achieve total biomass retention, high effluent quality, small footprint and low sludge production, but also significantly contribute to renewable bioenergy generation for the substitution of fossil fuel in power and heat production [1-4]. In particular, granular anaerobic membrane bioreactor (G-AnMBR), a hybrid anaerobic bioreactor incorporating granular technology with membrane based separation, offers a promising approach compared to the conventional anaerobic membrane bioreactor (C-AnMBR) predominantly in the form of continuous stirred tank reactor configuration. The competitive advantages of G-AnMBR include no requirement for mechanical mixing, significantly low energy demand and much more compact reactor design [5].
Contrary to conventional suspended growth bioflocs, anaerobic granules have regular and well-defined shape, strong structure, and good settling velocities, which can enable high biomass retention and withstand high strength wastewater and shock loadings, and produce biogas [6, 7]. The granule bed systems are usually featured with total biomass concentrations ranging from 20 to 40 g/L. All the biological reactions occurred within the dense granular sludge bed at the bottom of the anaerobic granular bioreactor. In a G-AnMBR, membrane module is not directly exposed to the bulk sludge and rather immersed in the sludge supernatant. Thus, the potential effects of suspended solids on membrane fouling can be reduced to some extent due to less apparent cake layer build-up and its consolidation compared to the C-AnMBR [8]. Garcia et al. [9] compared filtration performance of a G-AnMBR with a C-AnMBR when treating domestic wastewater. They observed that the G-AnMBR exhibited notably lower fouling rate, as the G-AnMBR demonstrated low concentrations of mixed liquor suspended solids (MLSS), and 50% less of SMP (soluble microbial products) concentration. In addition, Garcia et al. [10] reported lower fouling potential could be achieved in the G-AnMBR, which was attributed to the reduced solid and colloidal loading (by a factor of 10 and 3) on the membrane. Less fouling in G-AnMBR also ensured enhanced operation with increased fluxes and reduced gas sparging intensity.

However, recent research has shown that the integration of membrane into the granular systems could affect the hydraulics of granular sludge bed by eliminating the washout of fine floc sludge, thereby negatively impacting granular sludge properties [8, 11]. The accumulation of fine and colloidal flocs in sludge supernatant may also contribute to membrane fouling. In addition, at high liquid and biogas upflow velocity,
vigorous up and down movements of granules may break granules, resulting in granules fragmentation due to the high shear force [12-14]. It is essential to seek for strategies to maintain the quality of granules for long-term operation of submerged G-AnMBR, since the integrity of the anaerobic granules determines the efficiency and stability of anaerobic biological treatment and guarantees the sludge supernatant quality for controlling fouling propensity.

The low cost polyurethane sponge has been considered as an ideal attached growth mobile media in many aerobic submerged MBR studies to improve overall system performance due to its high specific surface area and internal porosity, light weight and high stability to hydrolyse [15]. Guo et al. [16] indicated sponge addition could significantly enhance the treatability of a conventional submerged membrane bioreactor, resulting in 2-time increase in sustainable flux. Additionally, sponge addition into submerged MBR can effectively retain biomass and enhance the flocculation ability of sludge flocs, leading to better membrane fouling mitigation and better nutrient removal [17, 18]. Deng et al. [17] also reported that sponge media could positively modify the sludge flocs, reduce SMP and EPS (extracellular polymeric substances), and prevent cake layer formation and pore clogging, thereby alleviating membrane fouling.

As far as we know, the effects of sponge, as the inert material, on the enhancement of granular sludge characteristics, and membrane fouling mitigation in the G-AnMBR have yet to be investigated for domestic wastewater treatment. Thus, this study aimed to evaluate the overall performance of a sponge assisted-granular anaerobic membrane bioreactor (SG-AnMBR) and a conventional G-AnMBR (CG-AnMBR). Granule
properties (e.g. particle size distribution (PSD), SMP and EPS, sludge volume index (SVI), etc.), fouling propensity (e.g. transmembrane pressure (TMP), SMP and EPS of the mixed liquor and cake layer, and foulants) and biogas production were also assessed.

2. Materials and methods

2.1. Wastewater

The lab scale experiments were conducted using synthetic wastewater to simulate domestic wastewater just after primary treatment. The synthetic wastewater is comprised of organics and macronutrients such as glucose, ammonium sulphate, potassium dihydrogen phosphate, and trace nutrients. The synthetic wastewater composition was slightly modified based on the previous study of Deng et al. [17] to maintain COD: N: P = 100: 2: 1. The synthetic wastewater contains dissolved organic carbon (DOC) of 100 - 120 mg/L, chemical oxygen demand (COD) of 330 - 370 mg/L, ammonia nitrogen (NH\(_4^+\)-N) of 5.5 - 6.6 mg/L, nitrite nitrogen (NO\(_2^-\)-N) of 0 - 0.02 mg/L, nitrate nitrogen (NO\(_3^-\)-N) of 0.2 - 0.8 mg/L, and orthophosphate (PO\(_4^{3-}\)-P) of 3.1 - 3.6 mg/L. NaHCO\(_3\) or NaOH was utilized to adjust pH to 7.

2.2. Experimental setup and operating conditions:

A CG-AnMBR and a SG-AnMBR with the same effective working volume (3 L) were operated in parallel at 20 °C in a temperature controlled room. The anaerobic sludge (MLSS = 22.34 ± 0.41 g/L, MLVSS = 17.41 ± 0.38 g/L, SVI = 98.5 mL/g, Mean particle size = 58 µm, Temperature = 21 °C and pH = 7.5) was from the anaerobic digester of a wastewater treatment plant in Sydney and was acclimatized to synthetic
wastewater for 30 days until a stable treatment performance was reached. The two reactors were fed with identical acclimatized anaerobic sludge with MLSS of 20.50 ± 1.53 g/L in the reaction zone. A polyvinylidene (PVDF) hollow fiber membrane with a pore size of 0.22 μm and surface area of 0.06 m² was immersed in the mixed liquor at the settling zone of each reactor. A vacuum driven peristaltic pump was employed to feed influent into the upflow anaerobic granular sludge bioreactor (UAGB). The other suction pump was operated with an intermittent suction cycle of 8 min on and 2 min off to acquire permeate from the membrane module. The purpose of the on/off cycle was to relax membrane unit and prevent the membrane fouling. Porous polyester-urethane sponge cubes (dimensions: 2 mm × 2 mm × 2 mm), namely S28-30/90 R (density of 28–30 kg/m³ with 90 cells per 25 mm, Joyce Foam Products), were added into the UAGB of the SG-AnMBR together with the inoculated sludge, and sponge volume fraction was 10% working volume. The CG-AnMBR and SG-AnMBR were operated at a constant filtration rate of 5.3 L/m²h with hydraulic retention time (HRT) of 12 h till membrane was fouled. Upflow velocity of 3.2 m/h was maintained using internal recirculation. The membrane fouling was indicated by development of the normalized TMP, which was recorded by a pressure transmitter. When TMP reached 30 kPa, operation was terminated. For the purpose of measuring membrane fouling resistance, hollow fibre membrane was taken out for chemical cleaning using the following three steps: 6 h in 0.4% sodium hydroxide, 6 h in 0.5% citric acid, and 6 h in 0.8% sodium hypochlorite.

2.3. Analytical methods

DOC of the sample was measured using a DOC analyzer (Analytikjena Multi N/C 2000). The equal amount of granular sludge was collected at 3 sampling port at different
heights of the UAGB (Port 1: 20 cm, Port 2: 40 cm and Port 3: 60 cm height from the bottom) and mixed for analysis, in order to represent the overall properties of granular sludge. The analysis of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), settling velocity, zeta-potential were carried out according to Standard Methods [19]. Three sludge samples were taken each time and the average value was then calculated for measuring MLSS and granular biomass. The method suggested by Nguyen et al. [24] was used for determining attached biomass in sponge. Spectrophotometric method using Spectroquant Cell Test (NOVA 60, Merck) was used to measure NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and PO₄³⁻-P. PSD of granule sludge was determined by using the laser particle size analysis system Mastersizer Series 2000 (Malvern Instruments Ltd. UK) with a detection range of 0.02–2000 mm. D (0.1) (i.e. 10% of the volume distribution was below this value) was used to describe the colloidal and fine particle fractions. The sludge granules were also examined by Microscope BX41 (Olympus, Japan) using Image-Pro Plus software.

Membrane fouling resistance of the G-AnMBR was determined by the resistance-in-series model using the following two equations:

\[ J = \frac{\Delta P}{\mu R_T} \]  
\[ R_T = R_M + R_C + R_P \]

Where \( J \) is the permeation flux (m\(^3\)/m\(^2\)/h); \( \Delta P \) is the transmembrane pressure (Pa); \( \mu \) is the dynamic viscosity of the permeate (Pa s); \( R_T \) is total resistance (m\(^{-1}\)); \( R_M \) is the intrinsic membrane resistance (m\(^{-1}\)); \( R_C \) is the cake layer resistance (m\(^{-1}\)); and \( R_P \) is the pore blocking resistance (m\(^{-1}\)). The method described by Deng et al. [20] was adopted
The extraction and analysis of EPS and SMP of the sludge sample, cake layer and mixed liquor were performed using the methods suggested by Deng et al. [17]. Modified Lowry method (Sigma, Australia) and Anthrone-sulfuric acid method were adopted for further determination protein (EPS$_P$ and SMP$_P$) and polysaccharide (EPS$_C$ and SMP$_C$) concentrations of the extracted samples. The total SMP or EPS concentration was calculated as the sum of the protein and polysaccharide. Foulants attached on the surface of membrane was extracted based on the methods provided by Johir et al. [21]. The extracted samples were further analysed using size exclusion liquid chromatograph with organic carbon detector (LC-OCD), a TSK HW 50-(S) column and a 0.028 mol/L phosphate buffer for the qualitative examination of the hydrophilic and hydrophobic fractions of the membrane foulant.

Volatile fatty acids (VFAs), namely acetate acid, propionic acid, butyric acid, isobutyric acid, iso-valeric acid and n-valeric acid were extracted using methyl-tert-butyl ether (MTBE) for liquid-liquid extraction according to the methods reported by Banel and Zygmunt [22]. Six VFAs were further quantified by gas chromatogram mass spectrometry method (GC-MS TQ8040, Shimadzu, Japan) using an open tubular analytical column (VF-WAXms, Agilent, US). An injection port equipped with a 1 mm internal diameter (ID) liner operated in splitless mode (after 1 min, split ratio was 1:10) was maintained at a temperature of 230 °C. Temperature program started at 50 °C and was held for 5 min before ramping to 250 °C at 10 °C/min and was then held for 10 min.
Helium was a carrier gas operated at a flow rate of 2.05 mL/min. Electron impact ion source was set at 230 °C while the injection port and transfer line temperatures were held at 230°C. Mass spectrometer (MS) was operated in a selected ion monitoring (SIM) mode and in a full scan mode (m/z 15-550). Ions for detection of individual VFA in SIM mode were selected using the mass spectra of standards generated in SCAN mode. Biogas production was collected using a biogas sample bag and determined using a liquor displacement device. Biogas composition including CH₄, CO₂, H₂ and N₂ is determined using potable biogas analyzer (Biogas 5000, Geotech, UK).

3. Results and discussion

3.1. Organics and nutrient removal

Both AnMBRs achieved organics removal efficiency higher than 90%. More specifically, the SG-AnMBR demonstrated slightly higher removals of DOC (92.4 ± 2.2%) and COD (93.7 ± 1.7%) when compared to those of the CG-AnMBR (90.1 ± 0.9% and 90.8 ± 1.4 %, respectively). The relatively high organics removal efficiencies could be attributed to the influent COD contained the majority of readily biodegradable COD using glucose as the sole carbon source. The complete retention of all particulate and colloidal matters by membrane also contributed to the high organics removal [23].

In general, total nitrogen (TN) and PO₄³⁻-P removal in the CG-AnMBR was low, which was found to be 15.0 ± 4.1% and 17.6 ± 6.2%, respectively. However, higher removal efficiencies were observed in the SG-AnMBR (31.7 ± 6.8% for TN removal and 36.2 ± 7.9% for PO₄³⁻-P removal), which is in line with the findings in Nguyen et al. [24]. The results revealed that the addition of sponge could not only enhance the removal of organic matter but also encourage nutrient removal in the G-AnMBR.
3.2. Granular sludge properties

3.2.1. Granular sludge

The successful implementation of anaerobic granular bioreactor technology relies on its capacity to retain a dense granular sludge bed for efficient physical entrapment and biodegradation of particulate and dissolved organic substances [25]. The CG-AnMBR and SG-AnMBR have been operated for 25 and 55 days, respectively, when TMP reached up to 30 kPa. As can be seen from Table 1, at the end of experimental period, MLSS concentrations of granular sludge increased to 23.82 g/L and 21.30 g/L in the SG-AnMBR and the CG-AnMBR, corresponding to the growth rate ($\Delta$MLSS/$\Delta$t) of 0.060 g/L⋅d and 0.032 g/L⋅d, respectively. The higher biomass growth rate in the SG-AnMBR indicated that the sponge addition encouraged the growth of retained sludge agglomerates in the granular sludge bed. Furthermore, the SG-AnMBR also presented higher MLVSS concentration with 19.10 g/L than that of the CG-AnMBR (16.59 g/L). The biomass attached to the sponge was found at 1.28 ± 0.41 g/g sponge.

In addition, the granular sludge from the SG-AnMBR also presented superior settling properties. At the end of the operation, granular sludge from the SG-AnMBR had SVI of 20.1 mL/g with settling velocity varying from 17.5 to 32.5 m/h (Table 1). Compared to the settling properties of the seed sludge, reduced SVI and increased settling velocities indicated that the settling properties of granular sludge were enhanced in the SG-AnMBR. On the other hand, granular sludge in the CG-AnMBR exhibited higher SVI of 58.5 mL/g and lower settling velocity of 14.1-18.4 m/h than those of the seed sludge, suggesting the sludge settleability was deteriorated. Zeta potential of the
granular sludge in the SG-AnMBR (-13.8 mV) was found higher than those of the CG-AnMBR (-21.1 mV) and the seed sludge (-15.5 mV). With increased zeta potential, the negative charge of the flocs could be neutralized and form large sludge aggregates with better settling characteristics [8, 17]. Since the development of well settling granular sludge requires selective washout of flocculent sludge with poor immobilization properties, the complete retention of small and colloidal flocs in a G-AnMBR by membrane barrier eliminated the hydraulic selection pressure required for granular sludge with good settling capacities. In this case, the growth of dispersed sludge would predominately take place, resulting in the bulking type of sludge formed in the CG-AnMBR with poor settling properties [17]. However, sponge addition could somehow improve granular sludge properties of the SG-AnMBR, and further alleviate the deterioration of granular sludge settling properties.

Table 1.

3.2.2. Granules

Generally, it has been reported the formation of sludge aggregates on or over 500 μm could be considered as granules [26]. However, a few studies have regarded sludge particles with 160 μm or less as granules [27-29]. Abbasi and Abbasi [12] suggested that granules size could range from 100 μm to 5 mm while Zhang et al. [30] reported average granule size increased from 111 μm to 264 μm from a hybrid anaerobic granular system with internal hydraulic circulation. Thus, in this study, bioparticles over 100 μm was considered as granules since synthetic domestic wastewater with low organic loading rate of 0.53-0.59 kg COD/m³·d was used as the feed and relatively short operation time was adopted. As compared to the seed sludge, one-way shift to fine
particles was observed in the CG-AnMBR while bigger size granules tended to form in the SG-AnMBR (Fig. 1). Based on the PSD of the granular sludge, the SG-AnMBR presented granules with increased diameter, compared to those of the CG-AnMBR. Fig. 1 shows that the percentage of granules (>100 μm) was approximately 84% of the total granular sludge in the SG-AnMBR, which was almost two times to the corresponding value obtained from the CG-AnMBR (42.5%). As membrane functioned as an absolute barrier in the CG-AnMBR, fine sludge particles (<100 μm), such as colloidal flocs, macromolecules of SMP and non-settling particles, could not be effectively discharged and rather accumulated in the CG-AnMBR, presenting lower percentage of granules. In contrast, sponge addition could assist granular growth by immobilizing fine particles on or inside the sponge pores, contributing to larger fraction of granules.

Fig. 1.

Apart from the complete retention of fine sludge particles, granules breakage could be another explanation for the lower amount of granules in the CG-AnMBR. Normally, EPS in the sludge plays a vital role in the synthesis of anaerobic granules, and is crucial for integrating cells into granules and maintaining intact structure of the granules. At the end of experiment, both protein and polysaccharides amounts of EPS decreased by 81.1% and 77.1% in the CG-AnMBR, as compared to the seed sludge EPS (EPSₚ and EPSₛ: 20.2 and 6.9 mg/g VSS), respectively. Therefore, the significant decrease in EPS amount might indicate scattered, loosen and weaker structures of granules (Fig. S1 in supplementary information), meaning granule fragmentation and decrease in particle size, as well as SMP increase in the mixed liquor [8]. On the contrary, the stable EPS
production in the SG-AnMBR was observed with the average values of 28.8 and 8.6 mg/g VSS for \( \text{EPS}_p \) and \( \text{EPS}_c \), respectively. Therefore, the higher contents of EPS promoted granule growth in the SG-AnMBR. Additionally, the amount of SMP from the CG-AnMBR sludge granules (Protein: 25.1 mg/g VSS, polysaccharide: 8.2 mg/g VSS) were found approximately 7 times higher than those from the SG-AnMBR (3.2 mg/g VSS, and 1.1 mg/g VSS). Much lower SMP values of the SG-AnMBR confirmed the majority of proteins and polysaccharides existed as the part of the anaerobic granules. As a result, the sponge addition had profound impacts on the EPS production of the anaerobic granules, as well as the granules abundance, structure and stability.

3.3. Membrane fouling behaviour

3.3.1. TMP profile

Fig. 2 showed the membrane fouling profile indicated by TMP development in two G-AnMBRs. Both systems showed significant differences in TMP profiles. As for the CG-AnMBR, the increase in TMP with time was characterized by a gradual rise at 0.3 kPa/d from day 1 to day 15, and then a rapid increase at 2.4 kPa/d till membrane was severely fouled on day 25. On the other hand, TMP in the SG-AnMBR was maintained well below 6 kPa within the first 25 days of operation and reached 30 kPa on day 55, indicating a relatively lower fouling rate of 0.5 kPa/d compared to the averaged 1.2 kPa/d for the CG-AnMBR. The results revealed that the sponge addition could greatly reduce fouling rate and improve the filtration performance of the G-AnMBR

Fig. 2.
3.3.2. SMP and EPS of the mixed liquor in settling zone

Membrane fouling was often attributed to the accumulation of organics in or on the membrane in the form of EPS and SMP [31]. Studies have reported that EPS clog the membrane pores, promoting the formation of a strongly attached fouling layer on the membrane surface while SMP can be absorbed onto the membrane surface, thereby blocking its pores and forming a gel layer acting as a barrier for permeate flux during filtration [20, 32]. Since the membrane was submerged in the mixed liquor of the G-AnMBR settling zone, SMP and EPS of the mixed liquor in both G-AnMBRs were analysed in order to explain the relationship between the mixed liquor properties and membrane fouling. As shown in Fig. 3, averaged SMP concentration in the CG-AnMBR was 47.3 ± 7.6 mg/L, which is almost three times higher than the value obtained in the SG-AnMBR (15.9 ± 3.5 mg/L). The significantly higher SMP amount in the CG-AnMBR was due to the release of biopolymeric substances to the mixed liquor as a result of granule and floc breakage and cell lysis [33]. This observation was further supported with particle size analysis, and EPS analysis of the granular sludge in Section 3.2. The bound EPS in the sludge could also be dissolved/ hydrolyzed into small fractions by bacterial hydrolysis [31]. Their subsequent dissolution into the water phase could result in more SMP release from microbial aggregates into the mixed liquor [8].

Fig. 3.

EPS concentrations of both systems remained increasing (Fig. 3) with the MLSS build-up in the mixed liquor. The MLSS concentrations in both G-AnMBRs increased gradually throughout the experimental period. At the end of experiment, the MLSS
concentration in the CG-AnMBR reached up to 770.2 mg/L, which was nearly 3 times higher than that of the SG-AnMBR (260.2 mg/L). The build-up of MLSS in the mixed liquor was mainly due to the membrane’s complete retention of small and colloidal flocs that would be otherwise selectively washed out from the system. The EPS concentration averaged at 17.0 ± 6.2 mg/L (SG-AnMBR) and 24.5 ± 11.0 mg/L (CG-AnMBR), and peaked at 24.5 mg/L (SG-AnMBR) and 39.3 (CG-AnMBR) when TMP reached 30 kPa. In the SG-AnMBR, sponge addition could help to limit the suspended growth [17], thus significantly reducing SMP and EPS concentrations in the mixed liquor by the means of adsorption onto the sponge and biodegradation by the attached biomass of the sponge. In addition, well-balanced granular and attached growth provided a sound environment for granules growth in the SG-AnMBR. Thus, the biodegradation of organics occurs mainly within the granules and attached biomass of the sponge, limiting the dispersed growth of light flocs. Colloidal particles coming from the influent solids could therefore be physically adsorbed and retained in the thick and dense granule bed, preventing their impact on the fouling [10].

3.3.3. Analysis of fouling resistance, cake layer and foulants

The fouling resistance was calculated according to the resistance-in-series model and the results are shown in Table 2. The $R_T$ of SG-AnMBR and CG-AnMBR were $9.7 \times 10^{13}$ m$^{-1}$ and $19.7 \times 10^{13}$ m$^{-1}$, respectively, indicating sponge addition into SG-AnMBR reduced the $R_T$ by 50.7%, compared to the CG-AnMBR. Higher $R_P$ was also found for the CG-AnMBR compared to the SG-AnMBR, corresponding to $9.5 \times 10^{12}$ m$^{-1}$ and $4.6 \times 10^{12}$ m$^{-1}$, respectively. $R_C$ of the CG-AnMBR (18.7$\times 10^{13}$ m$^{-1}$) accounted for 94.9% of $R_T$, whereas the SG-AnMBR had much lower $R_C$ at $9.2 \times 10^{13}$ m$^{-1}$,
corresponding to 94.8% of $R_T$. The resistance caused by $R_C$ presented dominant proportion of total resistance for both systems. Hence, minimizing the cake formation is of great importance to lower the fouling propensity of the G-AnMBR.

Table 2.

Contrarily, pore clogging, due to particles or colloids with equal or smaller size than the membrane pores, contributed to small portion of fouling resistance. The results were consistent with the findings of Liu et al. [34] in which sludge cake formation was the main mechanism of membrane fouling in the G-AnMBR. Jeison et al. [35, 36] also reported that TMP and flux was mainly governed by cake formation. The higher cake layer resistance in the CG-AnMBR could be ascribed to higher MLSS concentration in the mixed liquor where membrane was immersed. Assisted by sponge, the SG-AnMBR demonstrated the efficient solids entrapment of the dense granular sludge bed and contained much reduced MLSS of the mixed liquor. Lin et al. [37] identified that the cake formation rate was significantly affected by colloidal and fine particle size $D (0.1)$ of PSD. $D (0.1)$ of the CG-AnMBR was 30.1 $\mu$m, which was much smaller than those of the SG-AnMBR (62.5 $\mu$m). Considering the denser structure and reduced back transport velocity of the fine flocs, Liu et al. [34] suggested that the greater amount of fine particles in the CG-AnMBR are more likely to deposit on the surface of membrane, which in turn facilitates a cake layer denser than that with larger particles. Therefore, the results proved the sponge addition could greatly alleviate membrane fouling mainly by reducing the cake layer formation and pore clogging.
The compositions of bound EPS and SMP of the cake layer from both reactors were also analysed and compared. As shown in Table 2, sponge addition could efficiently reduce EPS\textsubscript{P} and SMP production in the cake layer of the SG-AnMBR. Higher concentration of EPS\textsubscript{P} (12.1 mg/g cake layer) was found in the CG-AnMBR than that in the SG-AnMBR (10.7 mg/g cake layer), while minor difference could be observed on EPS\textsubscript{C} of the cake layer from both G-AnMBRs. The CG-AnMBR demonstrated higher concentrations of SMP\textsubscript{P} and SMP\textsubscript{C} in the cake layer (8.2 and 4.1 mg/g cake layer, respectively) compared to the SG-AnMBR (5.6 and 2.5 mg/g cake layer, respectively). These results implied EPS\textsubscript{P}, SMP (including SMP\textsubscript{p} and SMP\textsubscript{c}) on the surface of the membrane were responsible for the higher $R_C$ in the CG-AnMBR. At relatively high TMP, more EPS\textsubscript{P}, SMP\textsubscript{P}, and SMP\textsubscript{C} could be deposited onto the membrane surface due to the high drag force from the permeate pump. Furthermore, the endogenous decay or cell lysis at the bottom layer could result in the release of more EPS\textsubscript{P} and SMP due to more sludge cake accumulated on the membrane surface [20].

LC-OCD provides important information regarding the fraction of organic matter in foulants by dividing the total organics into hydrophobic and hydrophilic groups. The hydrophilic fraction can be further subdivided into biopolymers, humic substances, building blocks, low molecular weight (LMW) acids and LMW neutrals and acids. As can be seen from Table 3, hydrophilic organics mainly contributed to membrane fouling, in which biopolymer was regarded one of the major foulants [21]. The value of biopolymers for the CG-AnMBR was found twice higher (34.6%) as compared to that for the SG-AnMBR (17.1%). The higher biopolymer concentrations in the CG-AnMBR indicated more hydrophilic layers built up on the membrane surface [38]. Furthermore,
bridging between inorganic compounds and deposited biopolymers could encourage the formation of more compact and dense fouling layer, leading to severe fouling [39]. Greater amount of building blocks (17.0% vs. 13.9%) and LMW neutrals and acids (35.1% vs. 31.2%) were also found in the CG-AnMBR compared to the SG-AnMBR. Aryal et al. [40] reported that building blocks and LMW neutrals and acids were vital factors causing fouling and enhancing the formation of biopolymers on the surface of the membrane possibly through their assemblage. Nevertheless, the CG-AnMBR exhibited lower humic substances (10.5%) than the SG-AnMBR (31.3%). Since the building blocks were the breakup of humic substances, lower fraction of humic substances might be related to the higher amount of building blocks in foulants of the CG-AnMBR [38].

Table 3.

3.4. VFA and biogas production

VFA serves as the most important process indicator for biogas production from G-AnMBRs not only because it can significantly influence pH value of the reactor but also due to the fact that it is the vital intermediary substrate for the methane generation [41]. Approximately 75% of methane yield comes from decarboxylation of acetic acid (main component of VFA) and the rest 25% is from CO₂ and H₂ [42]. If existing in high concentrations, VFA can also cause significant pH drop and pose enormous stress on sensitive methane-producing bacteria, thus ultimately resulting in G-AnMBRs reactor acidification and low biogas production [43-46]. In this study, seven types of VFAs including acetic (C₂), propionic (C₃), iso-butyric (i-C₄), n-butyric (n-C₄), iso-valeric acid (i-C₅), n-valeric (C₅) and caproic acid (C₆) were monitored. The SG-AnMBR
exhibited much lower level of acetic acid with the average value of 3.5 ± 0.8 mg/L, while other acids were at undetectable level (Table 4). The results revealed that there was no VFA accumulation in the SG-AnMBR, and reactor acidification was rarely encountered over the operation time. Therefore, the sponge could help to maintain a well-functioning granular sludge bed and efficient VFA degradation. Therefore, sponge could help to maintain a well-functioning granular sludge bed and efficient VFA degradation.

In contrast, the CG-AnMBR demonstrated much higher VFA concentrations with an average value of 20.2 ± 2.7 mg/L (5.8 times higher than that of the CG-AnMBR). VFA accumulation was mainly attributed to the existence of acetic acid (67.4 ± 7.7%) in the mixed liquor. C3, i-C4, n-C4, i-C5 and n-C5 were also detected in the CG-AnMBR. The accumulation of intermediate products VFA might be related to the VFA release as a result of granule disintegration or deteriorated methanogenic process. The stability of methanogenesis process is the key to the efficient biogas production. Since methanogens are very sensitive to environmental factor (oxidation/reduction potential (ORP), pH, etc), any variations in the operating conditions may cause inhibition for biogas production. Average pH values were found at 7.3 ± 0.3 and 6.9 ± 0.2 in the SG-AnMBR and CG-AnMBR, respectively, even though pH was not controlled. In the CG-AnMBR, the higher VFAs concentrations were accompanied by lower values of pH [47]. The changes of the ORP were also recorded. The ORP value in the SG-AnMBR was -318.4 ± 8.9 mV, which was 58.9 ± 8.9 mV lower than that in the CG-AnMBR on average. Lower ORP favoured the survival and growth of methanogens, therefore enhancing the transformation of VFAs into CH4 [29].

Table 4.
The SG-AnMBR produced more biogas (486 ± 12 mL/d) than the CG-AnMBR (456 ± 9 mL/d) with similar methane and carbon dioxide composition in the biogas (69.8 and 26.5%, 67.5 and 28.1%, respectively) (Table 5). Very small amount of H₂ with 5 - 12 ppm was also detected in the biogas from both reactors. The CG-AnMBR achieved methane yield at 133.3 ± 5.3 mL CH₄ (STP)/g CODremoving, volume of methane produced at and 0 °C Standard Temperature and 1 atm Pressure). While the SG-AnMBR had higher methane yield of 156.3 ± 5.8 mL CH₄ (STP)/g CODremoving.

Table 5.

The methane yield from the SG-AnMBR represented around 50% of the optimal theoretical value of 318 mL CH₄ (STP)/g CODremoving. As it is reported that methane loss in the liquid phase from the anaerobic MBR could be as much as 30% and 50% at 35 °C and 15 °C, respectively [48], nearly half of degraded COD might convert to dissolved methane and lost. Considering the economic and environmental impacts, methane leakages have to be paid much attention to and minimized [49, 50]. The development of feasible and effective recovery process for dissolved methane is highly desired for the optimization of bioenergy recovery and minimization of greenhouse gas emissions to the atmosphere. The available recovery processes include biological oxidation of dissolved methane using down-flow hanging sponge reactor [51], removal of residual dissolved methane using degassing membrane [52] and post-treatment aeration to strip of AnMBR effluent [53].

4. Conclusions
This study showed that the sponge addition into G-AnMBR could not only improve organics and nutrient removal, but also retain superior granular sludge properties and enhance methane yield. In addition, the SG-AnMBR exhibited prolonged operation time due to effective fouling mitigation. Assisted by sponge, the SG-AnMBR showed lower SMP and EPS levels in settling zone mixed liquor, less EPS* and SMP production in the cake layer as well as much lower cake layer and pore clogging resistance compared to those of the CG-AnMBR. Fouling resistance analysis revealed that sponge addition could reduce the RT by 50.7% via decreasing both cake layer and pore logging resistance. Furthermore, LC-OCD analysis confirmed that lower biopolymers, LMW neutrals and acids and building blocks were presented in the SG-AnMBR foulant. Further research on microbiological analysis is needed to look into differences in microbiological population or differences in the evolution of microbiological population in both SG-AnMBR and CG-AnMBR. This work offers a useful performance enhancement and fouling control strategy that a certain sponge volume could be added into the UAGB during G-AnMBR process.

References


[33] C. Kunacheva Y.N.A. Soh, A.P. Trzcinski, D.C. Stuckey, Soluble microbial products (SMPs) in the effluent from a submerged anaerobic membrane bioreactor


Table titles

**Table 1.** Summary of sludge characteristics of seed sludge and granular sludge in G-AnMBRs.

**Table 2.** Fouling resistance and cake layer analysis for both G-AnMBRs.

**Table 3.** Organic fractions of membrane foulants based on LC-OCD analysis.

**Table 4.** VFAs concentrations in the CG-AnMBR and the SG-AnMBR.

**Table 5.** Biogas yield from the CG-AnMBR and the SG-AnMBR.
Table 1.
Summary of sludge characteristics of seed sludge and granular sludge in G-AnMBRs.

<table>
<thead>
<tr>
<th>Sludge properties</th>
<th>Seed sludge</th>
<th>Granular sludge (CG-AnMBR)</th>
<th>Granular Sludge (SG-AnMBR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSS (g/L)</td>
<td>20.50 ± 1.53</td>
<td>21.30 ± 0.91</td>
<td>23.82 ± 1.83</td>
</tr>
<tr>
<td>MLVSS (g/L)</td>
<td>16.21 ± 1.85</td>
<td>16.59 ± 1.28</td>
<td>19.10 ± 1.11</td>
</tr>
<tr>
<td>Zeta-potential (mV)</td>
<td>-15.5 ± 3.5</td>
<td>-21.1 ± 2.5</td>
<td>-13.8 ± 1.8</td>
</tr>
<tr>
<td>SVI (mL/g)</td>
<td>38.8 ± 4.8</td>
<td>58.5 ± 5.1</td>
<td>20.1 ± 4.2</td>
</tr>
<tr>
<td>Settling velocity (m/h)</td>
<td>15.51 - 25.42</td>
<td>14.1-18.4</td>
<td>17.5 - 32.5</td>
</tr>
</tbody>
</table>
Table 2.
Fouling resistance and cake layer analysis for both G-AnMBRs.

<table>
<thead>
<tr>
<th>Fouling resistance (m⁻¹)</th>
<th>CG-AnMBR</th>
<th>SG-AnMBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rₜᵃ</td>
<td>19.7 × 10¹³</td>
<td>9.7 × 10¹³</td>
</tr>
<tr>
<td>Rₖᵇ</td>
<td>18.7×10¹³</td>
<td>9.2×10¹³</td>
</tr>
<tr>
<td>Rₚᶜ</td>
<td>9.5×10¹²</td>
<td>4.6×10¹²</td>
</tr>
<tr>
<td>Rₘᵈ</td>
<td>5.7×10¹¹</td>
<td>5.1×10¹¹</td>
</tr>
<tr>
<td>SMP and EPS in the cake layer (mg/g cake layer)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPSₚᵉ</td>
<td>12.1</td>
<td>10.7</td>
</tr>
<tr>
<td>EPSₖᶠ</td>
<td>3.6</td>
<td>3.4</td>
</tr>
<tr>
<td>SMPₚᵍ</td>
<td>8.2</td>
<td>5.6</td>
</tr>
<tr>
<td>SMPₖʰ</td>
<td>4.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

ᵃ Rₜ = total fouling resistance, ᵇ Rₖ = cake layer resistance, ᶜ Rₚ = pore blocking resistance, ᵈ Rₘ = clean membrane resistance, ᵉ EPSₚ = protein concentration of extracellular polymeric substances, ᶠ EPSₖ = polysaccharides concentration of extracellular polymeric substances, ᵍ SMPₚ = protein concentration of soluble microbial products, ʰ SMPₖ = polysaccharides concentration of soluble microbial products.
Table 3. Organic fractions of membrane foulants based on LC-OCD analysis.

<table>
<thead>
<tr>
<th>Operating conditions</th>
<th>DOC (^{a}) dissolved</th>
<th>HOC (^{b}) Hydrophobic</th>
<th>CDOC (^{c}) Hydrophilic</th>
<th>Biopolymers</th>
<th>Humic substances</th>
<th>Building blocks</th>
<th>LMW neutrals and acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>G-AnMBRs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foulant</td>
<td>SG-AnMBR</td>
<td>5360 (100%)</td>
<td>508 (9.5%)</td>
<td>4852 (90.5%)</td>
<td>918 (17.1%)</td>
<td>1675 (31.3%)</td>
<td>743 (13.9%)</td>
</tr>
<tr>
<td>Foulant</td>
<td>CG-AnMBR</td>
<td>5373 (100%)</td>
<td>152 (2.8%)</td>
<td>5221 (97.2%)</td>
<td>1857 (34.6%)</td>
<td>565 (10.5%)</td>
<td>915 (17.0%)</td>
</tr>
</tbody>
</table>

\(^{a}\) DOC = dissolved organic carbon, \(^{b}\) HOC = hydrophobic organic carbon, \(^{c}\) CDOC = chromatographic dissolved organic carbon.
Table 4.
VFAs concentrations in the CG-AnMBR and the SG-AnMBR.

<table>
<thead>
<tr>
<th>VFA</th>
<th>CG-AnMBR</th>
<th>SG-AnMBR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mg/L)</td>
<td>Fraction of VFA (%)</td>
</tr>
<tr>
<td>C2\textsuperscript{a}</td>
<td>13.6 ± 2.6</td>
<td>67.4 ± 7.7</td>
</tr>
<tr>
<td>C3\textsuperscript{b}</td>
<td>1.4 ± 0.9</td>
<td>6.9 ± 4.8</td>
</tr>
<tr>
<td>i-C4\textsuperscript{c}</td>
<td>1.0 ± 0.6</td>
<td>5.2 ± 3.5</td>
</tr>
<tr>
<td>n-C4\textsuperscript{d}</td>
<td>0.9 ± 0.7</td>
<td>4.6 ± 3.7</td>
</tr>
<tr>
<td>i-C5\textsuperscript{e}</td>
<td>1.1 ± 0.8</td>
<td>5.6 ± 4.4</td>
</tr>
<tr>
<td>n-C5\textsuperscript{f}</td>
<td>2.2 ± 2.2</td>
<td>10.3 ± 9.3</td>
</tr>
<tr>
<td>C6\textsuperscript{g}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VT\textsuperscript{h}</td>
<td>20.2 ± 2.7</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a}C2=acetic acid, \textsuperscript{b}C3=propionic acid, \textsuperscript{c}i-C4=iso-butyric acid, \textsuperscript{d}C 4=butyric acid, \textsuperscript{e}i-C5=iso-valeric acid, \textsuperscript{f}C5=valeric acid, \textsuperscript{g}C6= caproic acid, \textsuperscript{h}VT=total volatile fatty acids.
Table 5.
Biogas yield from the CG-AnMBR and the SG-AnMBR.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SG-AnMBR</th>
<th>CG-AnMBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogas volume (mL/d)</td>
<td>486 ± 12</td>
<td>456 ± 9 mL/d</td>
</tr>
<tr>
<td>Methane yield (mL CH₄/g COD removed)</td>
<td>156.3 ± 5.8 at STP</td>
<td>133.3 ± 5.3 at STP</td>
</tr>
<tr>
<td>Methane (%)</td>
<td>69.8 ± 4.2</td>
<td>67.5 ± 4.8</td>
</tr>
<tr>
<td>Carbon dioxide (%)</td>
<td>26.5 ± 4.8</td>
<td>28.1 ± 4.5</td>
</tr>
<tr>
<td>Hydrogen (ppm)</td>
<td>9.2 ± 2.8</td>
<td>8.1 ± 3.1</td>
</tr>
</tbody>
</table>

^STP = volume of methane produced at and 0 °C Standard Temperature and 1 atm Pressure.
Figures captions

**Fig. 1.** Particle size distribution of seed sludge, and granular sludge for both G-AnMBRs.

**Fig. 2.** TMP profile of the CG-AnMBR and the SG-AnMBR over the experimental period.

**Fig. 3.** Variations of EPS and SMP concentrations in the settling zone of G-AnMBR at designated TMPs.
Fig. 1.
Fig. 2.
Fig. 3.
Highlights

- Sponge based G-AnMBR is comprehensively studied and evaluated.
- Sponge addition improves granule properties and enhances system performance.
- The SG-AnMBR exhibits less fouling propensity compared to the CG-AnMBR.
- The SG-AnMBR shows no VFA accumulation and yields more biogas.
- The SG-AnMBR presents less organic fractions within the membrane foulants.