Single-stage versus two-stage anaerobic fluidized bed bioreactors in treating municipal wastewater: Performance, foulant characteristics, and microbial community

Wu, Bing; Li, Yifei; Lim, Weikang; Lee, Shi Lin; Guo, Qiming; Fane, Anthony G.; Liu, Yu

2016

Wu, B., Li, Y., Lim, W., Lee, S. L., Guo, Q., Fane, A. G., et al. (2017). Single-stage versus two-stage anaerobic fluidized bed bioreactors in treating municipal wastewater: Performance, foulant characteristics, and microbial community. *Chemosphere*, 171,158-167.

<https://hdl.handle.net/10356/86622>

<https://doi.org/10.1016/j.chemosphere.2016.12.069>

© 2016 Elsevier Ltd. This is the author created version of a work that has been peer reviewed and accepted for publication by *Chemosphere*, Elsevier Ltd. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [<http://dx.doi.org/10.1016/j.chemosphere.2016.12.069>].

Downloaded on 26 Apr 2023 13:54:02 SGT

**Single-stage versus Two-stage Anaerobic Fluidized Bed Bioreactors in Treating
Municipal Wastewater: Performance, Foulant Characteristics, and Microbial
Community**

Bing Wu^{1,*}, Yifei Li^{2,3}, Weikang Lim¹, Shi Lin Lee³, Qiming Guo¹,

Anthony G. Fane^{1,3}, Yu Liu^{2,3,*},

¹ Singapore Membrane Technology Centre, Nanyang Environment and Water Research
Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One #06-08,
Singapore, 637141

² Advanced Environmental Biotechnology Centre, Nanyang Environment and Water
Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One
#06-08, Singapore, 637141

³ School of Civil and Environmental Engineering, Nanyang Technological University, 50
Nanyang Avenue, Singapore, 639798

Corresponding authors:

Bing Wu, wubing@ntu.edu.sg, Phone: 65-91258929, Fax: 65-67910756;

Yu Liu, cylu@ntu.edu.sg, Phone: 65-67905254, Fax: 65-67910676

Abstract

This study examined the receptive performance, membrane foulant characteristics, and microbial community in the single-stage and two-stage anaerobic fluidized membrane bioreactor (AFMBR) treating settled raw municipal wastewater with the aims to explore fouling mechanisms and microbial community structure in both systems. Both AFMBRs exhibited comparable organic removal efficiency and membrane performances. In the single-stage AFMBR, less soluble organic substances were removed through biosorption by GAC and biodegradation than those in the two-stage AFMBR. Compared to the two-stage AFMBR, the formation of cake layer was the main cause of the observed membrane fouling in the single-stage AFMBR at the same employed flux. The accumulation rate of the biopolymers was linearly correlated with the membrane fouling rate. In the chemical-cleaned foulants, humic acid-like substances and silicon were identified as the predominant organic and inorganic foulants respectively. As such, the fluidized GAC particles might not be effective in removing these substances from the membrane surfaces. High-throughput pyrosequencing analysis further revealed that *beta-Proteobacteria* were predominant members in both AFMBRs, which contributed to the development of biofilms on the fluidized GAC and membrane surfaces. However, it was also noted that the abundance of the identified dominant in the membrane surface-associated biofilm seemed to be related to the permeate flux and reactor configuration.

Key words: Anaerobic MBR; Bacterial diversity; Granular activated carbon; Membrane fouling; Scouring.

1. Introduction

With increasing concerns on the sustainability of wastewater-energy nexus, anaerobic membrane bioreactors (AnMBRs) have received growing attention because of potential energy recovery and sludge reduction compared with conventional aerobic MBRs [1,2]. These suggest the possibility to achieve energy self-sufficiency in AnMBRs [3]. However, membrane fouling in AnMBRs is still a major challenge for their wide applications, which may lead to high energy consumption and operating cost. Therefore, the effective control strategies of membrane fouling are needed for sustainable operation of AnMBRs. Generally, for pressure-driven cross-flow AnMBRs, a high cross-flow velocity is required to prevent the deposition of foulants on membranes, whereas bubbling through biogas recirculation has been practiced to mitigate membrane fouling in vacuum-driven submerged AnMBRs [2,4]. However, these two methods for fouling control in AnMBRs are energy intensive. For example, the energy demand associated with biogas recirculation for fouling control had been reported to be in the range of 0.69-3.41 kWh/m³ in submerged AnMBRs [5], which was even higher than that the energy consumption incurred in aerobic submerged MBRs (e.g. 0.5-1.0 kWh/m³) [6].

As an alternative, two-stage anaerobic fluidized-bed membrane bioreactor (AFMBR) has been proposed by Kim et al. [7], in which the first-stage is an anaerobic fluidized bed bioreactor (AFBR) for biosolids reduction and biodegradation of soluble organics by anaerobic bacteria grown on granular activated carbon (GAC), and the second stage is an AFMBR where membrane fouling is expected to be controlled through the scouring created by fluidized GAC. Importantly, the calculated two-stage AFMBR energy requirement was only about 0.028-0.227 kWh/m³, which was significantly lower than conventional aerobic/anaerobic MBRs and could be offset by the produced methane gas [7,8].

To further reduce costs and footprint associated with the construction and the maintenance of AFMBR, combining the two stages into a single reactor configuration has been examined, e.g. Gao et al. [9,10] proposed an integrated reactor in which the outer loop of the reactor served as an AFBR and the inner loop was considered as an AFMBR, both with GAC as carriers. Moreover, Bae et al. [11] also evaluated the performance of a single-stage AFMBR in treating synthetic wastewater, and found that its performance was comparable with the two-stage AFMBR in terms of organic removal and membrane performance. It has been believed that in the two-stage AFMBR, the first-stage reactor can retain organic solids and degrade soluble organic substances, leading to reduced potential foulants going into the second-stage AFMBR. However, such observations should be further verified with real municipal wastewater containing more refractory organic substances and biosolids. In addition, little information is currently available for transport of organic substances, characterization of membrane foulants and microbial community structure in the single-stage and two-stage AFMBR systems.

In this study, a series of experiments were concurrently conducted in the single-stage and two-stage AFMBR systems fed with settled municipal wastewater, with the focus on better understanding of membrane fouling mechanisms and membrane foulants characterization at different permeate fluxes. The profiles of microbial communities developed on the membrane and GAC surfaces, and in the suspension were also determined. It is expected that this study can shed lights on the ways to further reduce energy consumption of AFMBRs by integrating two-stage reactors into a single-stage AFMBR for treating real municipal wastewater.

2. Materials and Methods

2.1. Operating conditions of single-stage and two-stage AFMBRs

Figure 1 describes a schematic diagram of single-stage and two-stage AFMBRs. Raw sewage ($24 \text{ m}^3/\text{day}$) from Ulu Pandan Water Reclamation Plant, Singapore flew through a pilot clarifier before feeding to the single-stage and two-stage AFMBRs. The two-stage AFMBR consisted of a pilot-scale AFBR installed at the Ulu Pandan Water Reclamation Plant, Singapore and a lab-scale AFMBR. The pilot AFBR had a reactor volume of 2 m^3 and contained 250 kg of 10×30 mesh GAC (Calgon Carbon, USA). Anaerobic digested sludge (equivalent to $\sim 850 \text{ g}$ dry weight) from the Ulu Pandan Water Reclamation Plant digester was inoculated into the AFBR. The GAC and sludge were kept fluidized by recycling reactor effluent at an upflow velocity of 0.009 m/s . The HRT of the pilot AFBR was 2 h. The biogas production rate was $0.14 \pm 0.08 \text{ L/min}$. After 240 days of operation, the AFBR effluent was fed to the lab-scale second-stage AFMBR. The second-stage AFMBR had an effective reactor volume of 2.7 L , containing 450 g GAC at a size of $1\text{--}1.4 \text{ mm}$ (Calgon Carbon, USA). A polyvinylidene fluoride (PVDF) hollow fiber membrane module (pore size at $0.1 \text{ }\mu\text{m}$, GE, USA) with an area of 0.022 m^2 was submerged into the reactor. The liquid upflow velocity was fixed at 0.018 m/s to ensure that the fluidized GAC particles fully scoured the membrane surface. The reactor was operated at a HRT of 2 h (i.e., 32.4 L/day of feed, a total HRT of the two-stage AFMBR was 4 h) and a flux of 10, 20, and $30 \text{ L/m}^2 \text{ h}$, which was correspondent to the volumetric flowrate ratio of discharge effluent to permeate at 5, 2, and 1 respectively. At each tested permeate flux, a new membrane module was employed.

The lab-scale single stage AFMBR had the same reactor volume, GAC packing amount, membrane configuration, and liquid upflow velocity as the second-stage AFMBR in the two-stage system except it was inoculated with 1.5 g (dry weight) of anaerobic digested sludge from the Ulu Pandan Water Reclamation Plant digester. The single-stage AFMBR was operated at a HRT of 3 h (i.e., 21.6 L/day) and a flux of 5, 10, 20, and $30 \text{ L/m}^2 \text{ h}$, which was correspondent to the volumetric flowrate ratio of discharge effluent to permeate at 7, 3, 1, and

0.4, respectively. The permeate suction pressure and permeate flow rate were recorded via Labview (National Instruments, USA) installed on a computer. Both systems were operated at infinity SRT (i.e., no sludge removal except sampling) and a room temperature at $23\pm1^{\circ}\text{C}$. The difference of HRT for the two membrane reactors (2 h vs. 3h) ensured that the both reactors received similar soluble organic loading (Table S1) for a fair comparison. In addition, as this study focused on membrane fouling mechanisms, thus, biogas production of both lab-scale AFMBRs were not examined.

2.2. Analytical methods

Chemical oxygen demand (COD), total suspended solids (TSS), and volatile suspended solids (VSS) were determined according to Standard Methods [12]. pH was measured by a pH meter (Hanna, USA). After centrifugating sample (for feed and effluent samples) at $4,000\times g$ for 10 min, the supernatant of the samples were taken for soluble COD (sCOD) and extracellular polymeric substances (EPS, mainly includes polysaccharides and protein) measurement. The polysaccharides and protein were analyzed according to Dubois [13] and Lowry [14] method, respectively. The sample was filtered with a $0.45\ \mu\text{m}$ filter (Pall Corporation, USA), and the dissolved total organic carbon (TOC) were measured by a TOC analyzer (Shimadzu, Japan). Inorganic elements were measured using ICP-MS (Perkin-Elmer, USA). It is noted that the membrane used in this study was $0.1\ \mu\text{m}$, thus, sCOD and sTOC in the permeate were the soluble organics with sizes less than $0.1\ \mu\text{m}$.

2.3. Average membrane fouling rate and contributions of cake layer fouling, irreversible fouling and irremovable fouling to total fouling

Membrane fouling rate was calculated by dividing the difference between the final transmembrane pressure (TMP_t) and initial TMP (TMP_0) by the filtration time. Before experiments, the clean membrane resistance (R_m) was measured by filtrating distilled water.

The clean membrane resistance for each tested membrane module was around $2.5 \times 10^{11} \text{ m}^{-1}$. At the end of filtration experiment, the total membrane resistance (R_t) was calculated according to the Darcy's law ($R = \text{TMP}/\mu J$). After that, the membrane was physically washed using distilled water (5 min) to remove cake layers foulants. The resistance of the physically-cleaned membrane (defined as $R_{\text{irreve+irremo+mem}}$) was measured using distilled water at the employed flux. The hollow fibres were then washed with 0.1 M citric acid (20 min), followed by 0.1 M NaOH (20 min). The resistance of the chemically-cleaned membrane (defined as $R_{\text{irremo+mem}}$) was measured using distilled water at the employed flux. The cake layer fouling resistance was derived from the difference between R_t and $R_{\text{irreve+irremo+mem}}$. The irreversible fouling resistance was calculated based on the difference between $R_{\text{irreve+irremo+mem}}$ and $R_{\text{irremo+mem}}$. The irremovable fouling resistance was calculated based on the difference between $R_{\text{irremo+membrane}}$ and R_{mem} .

2.4. Liquid chromatography-organic carbon detection-organic nitrogen detection (LC-OCD-OND)

Size-exclusion LC-OCD-OND was used to quantify the major soluble organic fractions with different molecular sizes and chemical functions in a sample after filtration through a $0.45 \mu\text{m}$ hydrophilic filter [15]. An on-line purified mobile phase was delivered by an HPLC pump (S-100, Knauer, Berlin, Germany) at a flow rate of 1.1 mL/min to an autosampler (MLE, Dresden, Germany) and to the chromatographic column (TSK HW 50S, 3000 theoretical plates, Toso, Japan). A non-destructive, fixed wavelength UV- detector (UVD 254 nm, type S-200, Knauer, Berlin, Germany) was used as the first detector after chromatographic separation for analyzing organic carbon. For organic nitrogen detection, a side stream was diverted after UVD with a restricted flow rate 0.1 mL/min (back pressure-driven). The mobile phase consisted of 2.5 g/L KH_2PO_4 and 1.5 g/L $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (pH of 6.85). An acidification solution was prepared by mixing 4 mL of 85% o-phosphoric acid and

0.5 g potassium peroxodisulfate with 1 L of distilled water. The chemicals were purchased from Aldrich-Sigma, USA.

2.5. Excitation-emission matrix (EEM) fluorescence spectroscopy analysis

A fluorescence spectrophotometer (LS 55, Perkin Elmer Company, USA) was used for obtaining EEM spectra in the wavelength range of 230 and 550 nm. Excitation and emission slits were set at 10 nm with a scanning speed of 1000 nm/min. Fluorescence regional integration (FRI) was applied to describe the volumetric percentage of a given component following the method proposed by Chen and his colleagues [16].

2.6. Microbial community analysis

The biofilm samples were collected from the fluidized GAC particles and fouled membrane surfaces. The genomic DNA of the microbial community in the biofilm samples and reactor effluents was extracted by FastDNA® SPIN kit (MP Biomedicals, USA). The variable V1-V3 region of the 16S rRNA gene was sequenced by Illumina (Research and Testing Laboratory, USA) using primers 357wF and 785R. Sequencing results were analysed by Mothur 1.36.1 software using the standard de novo operational taxonomic unit (OTU) approach.

3. Results and Discussion

3.1. Reactor performances in single-stage and two-stage AFMBRs

The feed, effluent, and permeate quality of the single-stage and two-stage AFMBR systems are presented in Table 1. As shown in Figure 1, the effluent samples with potential foulants were taken from the upper part of the AFMBRs. In order to operate the two systems under the similar organic loading, but with different membrane permeate fluxes, a portion of AFMBR effluent was discharged accordingly (Table S1). Nevertheless, it was found that more than

97% of total COD (TCOD) and almost 100% of suspended solids were removed in both AFMBRs, indicating that eliminating first-stage AFBR could not significantly affect the overall performance. In fact, similar observation was also reported by Bae et al. [11] with a mixture of acetate and propionate as feed.

Furthermore, it was found that about 33% of sCOD and 35% of TOC could be removed likely by biosorption and biodegradation, whereas another 32% of sCOD and 40% of TOC were rejected by membrane separation in the single-stage AFMBR. In contrast, the contribution of biosorption and biodegradation to overall soluble organic substances removal in the two-stage AFMBR (49% of sCOD and 55% of TOC) was slightly higher than those in the single-stage AFMBR. This may be attributed to (1) more attached biomass in the two-stage AFMBR due to higher total GAC dosage compared to that in the single-stage AFMBR; (2) slightly longer HRT (4 h) in the two-stage AFMBR compared to the single-stage AFMBR (3 h). On the other hand, it was found that more soluble organic substances were removed by membrane separation in the single-stage AFMBR than in the two-stage AFMBR (i.e. 32% vs. 26% for sCOD and 40% vs. 25% for TOC). According to the LC-OCD and EEM analyses (Figures S1 and S2), the compositions of the soluble organic substances in the effluents from the single-stage AFMBR and the 2nd-stage of the two-stage AFMBR were highly similar, in which biopolymers (i.e., microbial by products-like substances) and humic-like substances were predominant soluble organic substances.

In the two-stage AFMBR system, the first-stage AFBR could remove 67% of TSS, 58% of VSS, 49% of TCOD, 31% of sCOD, and 32% of TOC respectively, while the attached-biofilms on the GAC particles in the second-stage AFMBR further contributed to the removal of residual soluble organics (i.e., 18% of sCOD and 13% of TOC). However, on the average, the higher solid contents in the second-stage AFMBR effluent were observed compared to its feed (i.e., the effluent of the AFBR). This is possibly due to the accumulation of the non-

biodegradable solid substances which passed through the first-stage AFBR and was rejected by the membrane filtration in the second-stage AFMBR.

3.2. Membrane performances in single-stage and two-stage AFMBRs

The TMP-time profiles at various permeate fluxes in both systems are shown in Figure 2. At each tested permeate flux, a new membrane module was employed. Apparently, in both AFMBRs, when the membrane was operated at 5, 10, and 20 L/m²h, the corresponding TMP profiles appeared to follow a three-stage transient pattern. Such a TMP profile had been commonly observed in aerobic MBRs operated at a sustainable permeate flux. This phenomenon was likely due to the initial pore blockage of the membrane leading to a sudden increase of TMP, followed by further progressive cake formation and pore blockage on/in membranes [17]. However, at the highest flux of 30 L/m²h, the TMP jumped to above 20 kPa within a few days in a quasi-linear pattern, i.e. the tested flux exceeded the threshold flux (i.e., critical flux) range in both AFMBRs.

It should be noted that both AFMBR exhibited comparable membrane performances at the fluxes below 20 L/m²h, implying that HRT could not have significant effect on the membrane performance. This observation was consistent with the previous study conducted at 6-9 L/m²h with a mixture of acetate and propionate as the feed [11]. However, at the higher flux of 30 L/m²h, the fouling appeared much server in the single-stage than in the two-stage AFMBR probably because of more soluble organic substances in the single-stage AFMBR (Table 1).

The comparison of membrane fouling rate shows that increasing permeate flux did not significantly result in higher membrane fouling rates in both AFMBRs at the fluxes lower than 20 L/m²h (Table S2). However, further increasing the flux from 20 to 30 L/m²h led to a sharp increase in the membrane fouling rate by 40 times in both single-stage and two-stage AFMBRs. In AFMBRs, through fluidization of the GAC particles, much more energy can be

imparted to the membrane surface instead via the flowing liquid [18]. Accordingly, the interaction of the fluidized GAC particles with the membrane surface prevented deposition of the foulants from membrane surface. At the lower fluxes, the GAC scouring force was stronger than the interaction force of the foulants with the membrane. As a result, the observed membrane fouling was insignificant. However, when the flux was beyond a certain level, the situation was reversed, i.e. the deposition of foulants became faster than they were removed from the membrane surfaces through the GAC scouring. Consequently, an accelerating development of fouling layer was observed.

Figure 3 further shows that the formation cake layer tended to be the main cause of the observed membrane fouling at an increased permeate flux, while less irremovable fouling was observed in both AFMBRs. At a lower permeate flux, the foulants tended to form relatively loose cake layer, which could be removed by the scouring force induced by the fluidized GAC particles. On the contrary, at a higher permeate flux, the GAC scouring force might not be strong enough to remove the foulants trapped in the denser cake layer [19]. However, such denser cake layers in turn served as a secondary filtration later to prevent the further deposition of fine foulants, leading to less irreversible and irremovable fouling [20].

Apparently, at the same permeate flux, the cake layer membrane fouling in the single-stage AFMBR contributed more significantly than that in the 2nd stage of two-stage AFMBR. In contrast, the irremovable fouling in the single-stage AFMBR was less than that in the 2nd stage of two-stage AFMBR at the same membrane flux. This hints that the formed fouling layers that were not removed by GAC scouring in the single-stage AFMBR could be readily removed by further physical cleaning. This observation provides a possibility to effectively control membrane fouling by additional physical cleaning protocols (such as periodically backwashing or flushing) or by improved GAC scouring efficiency (such as increasing GAC

packing density and particle size [19,22]) during long-term operation of single-stage AFMBRs.

Furthermore, the major cake layer and irreversible foulants that were physically and chemically recovered from fouled membrane respectively were characterized. The cake layer foulant characteristics are listed in Table 2. With increasing permeate flux, the accumulation rate of the deposited foulants (i.e., TSS) on the membranes and the ratio of VSS (i.e., microorganisms) to TSS tended to increase. This could be ascribed to the accumulated organic substances, which promoted biofilm proliferation. The results suggest that the increased TSS and VSS concentrations accelerated membrane fouling (Table S2), similarly to the observation in conventional anaerobic MBRs [21,22].

As for the soluble foulants, the TOC and EPS accumulation rates appeared to slightly increase when the flux increased from 5 to 20 L/m² h, but a sharp increase in TOC and EPS accumulation was observed when the flux was further increased to 30 L/m² h. Such a pattern was in line with the observed membrane fouling rate, i.e. the deposition of the soluble organic substances on membrane determined the membrane performance as often reported in MBRs [20,23]. In addition, the polysaccharides/protein ratio of the foulants in the single-stage AFMBR was found to be more or less lower than those in the two-stage AFMBR at the same permeate flux. Although proteins are more hydrophobic and more easily to attach on membrane surface [22], in this study, membrane fouling rate was more correspondent with total EPS amount, rather than polysaccharides/protein ratio. This is contrary to some studies on AnMBRs [22,24], possibly attributing to different materials and pore size of the used membranes [25].

To further identify the comprehensive compositions of the soluble foulants, LC-OCD and EEM analyses were performed. According to the LC-OCD analysis (Figure 4), more

accumulation of the soluble organic substances in the cake layers appeared in the improved permeate flux, which was strongly consistent with the TOC and EPS data. Especially, in both AFMBRs, the accumulation rate of the biopolymers was positively correlated with the membrane fouling rate (Figure S3). This is in the good agreement with the findings reported in the literature [26,27].

Figure 5 further shows the ratio of each component in the soluble foulants derived from the cake layer for both AFMBRs. Compared to the composition of soluble organics in the AFMBR effluent (Figure S1), less biopolymers and more humic acids were found at the flux of 5 L/m² h in the single-stage AFMBR and at the flux of 10 L/m² h in the 2nd stage of two-stage AFMBRs. This implies that the fluidized GAC particles might remove biopolymers due to their loose-nature at a lower flux. This finding was in accordance with the observation by Jin et al. [28] that less biopolymers were accumulated in the cake layers formed on the membrane in the presence of biocarriers at a flux of 9.13 L/m² h. It was also found that humic substances tended to be accumulated on the membranes possibly due to their strong affinity to the membrane surface. Moreover, with increasing permeate flux, the biopolymers became the predominant soluble foulants in the cake layers for both AFMBRs. It is likely that under stronger permeation force, biopolymers-membrane and biopolymers-biopolymers interactions were not easily broken down by the scouring force induced by the fluidized GAC.

In addition, EEM spectra offer alternative insights into the detailed compositions of soluble foulants, while FRI analysis of EEM spectra (Figure 6) further revealed that the humic substances in the soluble cake layer foulants was reduced with increasing the permeate flux in both AFMBRs. In contrast, the microbial by-product-like substances (i.e., biopolymers) tended to be more dominant at a higher permeate flux. These observations are well supported by the LC-OCD data.

As can be seen in Figure 7, humic substances were the major components in the irreversible foulants for both AFMBRs, regardless of permeate flux. With the increase in permeate flux, the content of humic substances slightly decreased, accompanied with the increase in microbial by-products-like substances. This may be attributed to the formation of biopolymers-biopolymers matrix in the cake layers at higher flux, which in turn protected the membrane surface from the attachment of humic substances.

Previous studies have illustrated Mg, Al, Fe, Ca and Si had significant effects on the formation of dense gel layer by bridging bacterial cells and organic substances [25,29]. Figure 8 indicated that Ca and Si were predominant inorganic substances in the irreversible foulants, and the permeate flux had a negligible effect on inorganic substances accumulation rate in the irreversible foulants at a flux lower than 20 L/m² h in both AFMBRs. However, sharply increased inorganic accumulation rate was observed at the flux of 30 L/m² h. In particular, with improving permeate flux in both AFMBRs, the content of silica was increased in the irreversible foulants, following a similar pattern to the microbial by-products-like substances (i.e., biopolymers). The formation of irreversible fouling could be explained by the fact that the silica element behaved as a major chelating agent to bind with microbial products-like substances to form non-porous cake layers which could not be easily removed by the fluidized GAC [25].

3.3. Bacterial community development in single-stage and two-stage AFMBRs

The fixed biomass was carefully collected from the fluidized GAC particles at three locations (bottom, medium and top of the fluidized bed) and the fouled layers of the membranes operated at fluxes of 20 and 30 L/m² h in both AFMBR systems. It was found that bacterial 16S rRNA sequences were related to eight different phyla: *Acidobacteria* (1.0-2.4%), *Actinobacteria* (1.0-7.7%), *Bacteroidetes* (5.5-13.3%), *Chloroflexi* (1.9-6.7%),

Proteobacteria (42.2-68.5%), *Verrucomicrobia* (0.7-2.7%), *Firmicutes* (1.5-6.4%) and unclassified bacteria (14.6-29.6%). *Proteobacteria* and *Bacteroidetes* showed higher abundance in both bulk sludge and biofilm samples, similar to the previous findings in anaerobic reactors [30-32].

At the class level, it was found that *beta-proteobacteria* (especially *Rhodocyclaceae* family), was predominant in the bulk sludge and biofilms on the fluidized GAC and membranes in both AFMBR reactors (Figures 9a and 10a). Notably, in each AFMBR reactor, the abundance of *beta-Proteobacteria* in the biofilms on the fluidized GAC and membranes (at a flux of 20 L/m² h) was significantly higher than that in the bulk sludge, which implies that these bacteria were mainly responsible for the formation of biofilms. In addition, some predominant bacterial species in the biofilms were associated with the reactor configuration and properties of GAC and membrane. For instance, in the single-stage AFMBR, the respective abundances of *delta-proteobacteria*, *Flavobacteria*, and *Acidobacteria_Gp3* in the biofilms on the fluidized GAC were slightly higher than those in the bulk sludge. While, in the two-stage AFMBR, the abundances of *alpha-Proteobacteria* and *Acidobacteria_Gp3* in the GAC biofilm were moderately higher than those in the bulk sludge. In the biofilms formed on the membranes, slightly higher abundances of *Sphingobacteria* and *Flavobacteria* were observed, compared to those in bulk sludge of the AFMBRs. Although *Clostridia* (*Firmicutes* phylum) were reported to be responsible for the formation of heterogeneous biofilms in an anaerobic process [33], this study suggested that the abundances of *Acidobacteria*, *Proteobacteria*, and *Bacteroidia* phylotypes could compete with *Clostridia* and largely contributed to the development of biofilms on the fluidized GAC and membranes at a flux of 20 L/m² h [30]. However, some species, such as *Actinobacteria*, *gamma-proteobacteria*, *Verrucomicrobiae*, tended to not attach onto the GAC and membrane surfaces in both AFMBRs operated at the flux of 20 L/m² h.

At the genus level, the microbial communities on the fluidized GAC and membranes as well as in the bulk sludge were highly diversified and composed of more than 900 OTUs. Most of the OTUs were detected at very low abundance, while only a few species were present at a abundance higher than 0.5%, such as *Longilinea*, *Novosphingobium*, *Methyloversatilis*, *Sulfuritalea*, *Desulfovibrio*, *Geobacter*, *Desulfomonile*, *Smithella*, *Sulfuricurvum*, *Methylococcus* (Figure 9b and 10b). These suggest that the dominant microbial species seemed similar in both AFMBRs.

Furthermore, in the biofilms on the membrane surfaces, the abundances of some dominant bacteria seemed to be flux-dependent (Figure 9). For example, with increasing the permeate flux, the abundance of *beta-proteobacteria* increased in the single-stage AFMBR, but decreased in the two-stage AFMBR. This may be due to the following reasons: (i) the systems at a flux of 20 L/m² h were operated for longer time than those at 30 L/m² h. This could allow proliferation of slowly-growing bacteria on the membrane surfaces; (ii) the GAC scouring was able to remove loosely-attached biofilm at a flux of 20 L/m² h (i.e., sub-sustainable flux).

On the other hand, in the biofilms on the GAC, the abundances of the dominant bacteria appeared to be also associated with the reactor configuration (Figure 10). Compared to the single-stage AFMBR, higher abundances of *alpha-* and *beta-proteobacteria* and lower abundance of *delta-proteobacteria* were found in the biofilms on the GAC in the two-stage AFMBR. However, in both AFMBR reactors, the abundance of *Anaerolineae* in the GAC biofilms increased with the fluidization bed height, while the abundances of *Actinobacteria*, *Sphingobacteria*, *alpha-Proteobacteria*, *gamma-Proteobacteria*, and *Clostridia* tended to decrease with the fluidization bed height. This may be associated with combined effects of (i) decreased organic concentration with the fluidization bed height as the wastewater was fed into the reactor from the bottom part, promoting the bacterial growth at lower nutrient levels

and (ii) the bigger size of the fluidized GAC particles at the bottom due to size segregation, i.e. the higher scouring force was expected at the bottom [34].

4. Conclusions

This study investigated the behaviours of membrane filtration at different permeate fluxes and bacterial community structures in the single-stage and two-stage AFMBRs with the aim to compare the performances of the two systems and explore membrane fouling mechanism. The following conclusions can be drawn:

- (1) More than 97% of total COD and almost 100% of suspended solids could be removed in the two systems, indicating the unnecessary of the first-stage AFBR in the two-stage AFMBR system for treating municipal wastewater.
- (2) The overall performances of the two AFMBR systems at a low flux were comparable in terms of membrane performance and fouling development. The formation of cake layer was the main contributor to the observed membrane fouling, whereas irremovable fouling was less important in the single-stage AFMBR compared to those in the two-stage AFMBR.
- (3) The foulants characteristics exhibited significant difference at a low flux, but were found to be similar at a high flux in both AFMBR systems.
- (4) In both AFMBR systems, *beta-Proteobacteria* were predominant. Although the reactor configuration slightly influenced the abundance of the dominant bacteria in the bulk sludge as well as in the biofilm, the shifts in abundance of these dominant bacteria indeed followed a similar pattern along the fluidization bed height.

Consequently, it was demonstrated in this study that a single-stage AFMBR instead of a two-stage AFMBR would be a promising option for the treatment municipal wastewater.

Acknowledgements

The authors are grateful to Prof. Perry McCarty from Stanford University and Prof. Jaeho Bae and Prof. Jeonghwan Kim from Inha University for their valuable discussion. This research grant (1102-IRIS-02-01) is supported by the Singapore National Research Foundation under its Environment and Water Research Programme and administered by PUB. The Economic Development Board (EDB) is also acknowledged for funding the Singapore Membrane Technology Centre (SMTC), Nanyang Technological University.

References:

- [1] G. Skouteris, D. Hermosilla, P. Lopez, C. Negro, A. Blanco, Anaerobic membrane bioreactors for wastewater treatment: A review, *Chem. Eng. J.* 198 (2012) 138-148.
- [2] D.C. Stuckey, Recent developments in anaerobic membrane reactors, *Bioresource Technol.* 122 (2012) 137-148.
- [3] P.L. McCarty, J. Bae, J. Kim, Domestic Wastewater Treatment as a Net Energy Producer-Can This be Achieved?, *Environ. Sci. Technol.* 45 (2011) 7100-7106.
- [4] A.L. Smith, L.B. Stadler, N.G. Love, S.J. Skerlos, L. Raskin, Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review, *Bioresource Technol.* 122 (2012) 149-159.
- [5] I. Martin, M. Pidou, A. Soares, S. Judd, B. Jefferson, Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment, *Environ. Technol.* 32 (2011) 921-932.
- [6] P. Krzeminski, J.H.J.M. van der Graaf, J.B. van Lier, Specific energy consumption of membrane bioreactor (MBR) for sewage treatment, *Water Sci. Technol.* 65 (2012) 380-392.
- [7] J. Kim, K. Kim, H. Ye, E. Lee, C. Shin, P.L. McCarty, J. Bae, Anaerobic Fluidized Bed Membrane Bioreactor for Wastewater Treatment, *Environ. Sci. Technol.* 45 (2011) 576-581.
- [8] C. Shin, P.L. McCarty, J. Kim, J. Bae, Pilot-scale temperate-climate treatment of domestic wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR), *Bioresource Technol.* 159 (2014) 95-103.
- [9] D.W. Gao, Q. Hu, C. Yao, N.Q. Ren, Treatment of domestic wastewater by an integrated anaerobic fluidized-bed membrane bioreactor under moderate to low temperature conditions, *Bioresource Technol.* 159 (2014) 193-198.
- [10] D.W. Gao, Q. Hu, C. Yao, N.Q. Ren, W.M. Wu, Integrated anaerobic fluidized-bed membrane bioreactor for domestic wastewater treatment, *Chem. Eng. J.* 240 (2014) 362-368.
- [11] J. Bae, C. Shin, E. Lee, J. Kim, P.L. McCarty, Anaerobic treatment of low-strength wastewater: A comparison between single and staged anaerobic fluidized bed membrane bioreactors, *Bioresource Technol.* 165 (2014) 75-80.
- [12] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC. (1998).

- [13] M. Dubois, K. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for the determination of sugars and related substances, *Anal. Chem.* 28 (1956) 350-356.
- [14] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.* 193 (1951) 265-275.
- [15] S.A. Huber, A. Balz, M. Abert, W. Pronk, Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography - organic carbon detection - organic nitrogen detection (LC-OCD-OND), *Water Res.* 45 (2011) 879-885.
- [16] W. Chen, P. Westerhoff, J.A. Leenheer, K. Booksh, Fluorescence excitation - Emission matrix regional integration to quantify spectra for dissolved organic matter, *Environ. Sci. Technol.* 37 (2003) 5701-5710.
- [17] P. Le-Clech, V. Chen, T.A.G. Fane, Fouling in membrane bioreactors used in wastewater treatment, *J. Membrane Sci.* 284 (2006) 17-53.
- [18] J.W. Wang, B. Wu, S.L. Yang, Y. Liu, A.G. Fane, J.W. Chew, Characterizing the scouring efficiency of Granular Activated Carbon (GAC) particles in membrane fouling mitigation via wavelet decomposition of accelerometer signals, *J. Membrane Sci.* 498 (2016) 105-115.
- [19] M. Saleem, L. Alibardi, M.C. Lavagnolo, R. Cossu, A. Spagni, Effect of filtration flux on the development and operation of a dynamic membrane for anaerobic wastewater treatment, *J. Environ. Manage.* 180 (2016) 459-465.
- [20] B. Wu, T. Kitade, T.H. Chong, T. Uemura, A.G. Fane, Role of initially formed cake layers on limiting membrane fouling in membrane bioreactors, *Bioresource Technol.* 118 (2012) 589-593.
- [21] Q.R. Dong, W. Parker, M. Dagnew, Long term performance of membranes in an anaerobic membrane bioreactor treating municipal wastewater, *Chemosphere* 144 (2016) 249-256.
- [22] Z. Huang, S.L. Ong, H.Y. Ng, Submerged anaerobic membrane bioreactor for low-strength wastewater treatment: Effect of HRT and SRT on treatment performance and membrane fouling, *Water Res.* 45 (2011) 705-713.
- [23] F.G. Meng, F.L. Yang, B.Q. Shi, H.M. Zhang, A comprehensive study on membrane fouling in submerged membrane bioreactors operated under different aeration intensities, *Sep. Purif. Technol.* 59 (2008) 91-100.
- [24] H.J. Lin, K. Xie, B. Mahendran, D.M. Bagley, K.T. Leung, S.N. Liss, B.Q. Liao, Sludge properties and their effects on membrane fouling in submerged anaerobic membrane bioreactors (SAnMBRs), *Water Res.* 43 (2009) 3827-3837.
- [25] X.Y. Zhang, Z.W. Wang, Z.C. Wu, T.Y. Wei, F.H. Lu, J. Tong, S.H. Mai, Membrane fouling in an anaerobic dynamic membrane bioreactor (AnDMBR) for municipal wastewater treatment: Characteristics of membrane foulants and bulk sludge, *Process Biochem.* 46 (2011) 1538-1544.
- [26] C. Fu, X. Bi, H.Y. Ng, Effects of bio-carriers on membrane fouling mitigation in moving bed membrane bioreactor, *J. Membrane Sci.* 499 (2016) 134-142.
- [27] M.A.H. Johir, S. Vigneswaran, A. Sathasivan, J. Kandasamy, C.Y. Chang, Effect of organic loading rate on organic matter and foulant characteristics in membrane bioreactor, *Bioresource Technol.* 113 (2012) 154-160.
- [28] L. Jin, S.L. Ong, H.Y. Ng, Fouling control mechanism by suspended biofilm carriers addition in submerged ceramic membrane bioreactors, *J. Membrane Sc.* 427 (2013) 250-258.
- [29] Z.W. Wang, Z.C. Wu, S.J. Tang, Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy, *Water Res* 43 (2009) 1533-1540.

- [30] J.X. Ma, Z.W. Wang, X.X. Zou, J.J. Feng, Z.C. Wu, Microbial communities in an anaerobic dynamic membrane bioreactor (AnDMBR) for municipal wastewater treatment: Comparison of bulk sludge and cake layer, *Process Biochem.* 48 (2013) 510-516.
- [31] Z.F. Xie, Z.W. Wang, Q.Y. Wang, C.W. Zhu, Z.C. Wu, An anaerobic dynamic membrane bioreactor (AnDMBR) for landfill leachate treatment: Performance and microbial community identification, *Bioresource Technol.* 161 (2014) 29-39.
- [32] X.Q. Shi, K.K. Ng, X.R. Li, H.Y. Ng, Investigation of Intertidal Wetland Sediment as a Novel Inoculation Source for Anaerobic Saline Wastewater Treatment, *Environ. Sci. Technol.* 49 (2015) 6231-6239.
- [33] N. Fernández, E.E. Díaz, R. Amils, J.L. Sanz, Analysis of microbial community during biofilm development in an anaerobic wastewater treatment reactor, *Microb. Ecol.* 56 (2008) 121-132.
- [34] J. Wang, F. Zamani, A. Cahyadi, J.Y. Toh, S. Yang, B. Wu, Y. Liu, A.G. Fane, J.W. Chew, Correlating the hydrodynamics of fluidized granular activated carbon (GAC) with membrane-fouling mitigation *J. Membrane Sci.* 510 (2016) 38-49.

Figure 1. A schematic diagram of single-stage and two-stage AFMBRs.

Figure 2. TMP developments at different permeate fluxes in the single-stage (a) and two-stage (b) AFMBRs.

Figure 3. Contributions of cake layer fouling, irreversible fouling and irremovable fouling to total fouling.

Figure 4. Compositions of the soluble cake layer foulants in the single-stage AFMBR (a) and two-stage AFMBR (b) analyzed by LC-OCD.

Figure 5. Component ratio of the soluble cake layer foulants analyzed by LC-OCD.

Figure 6. Component ratio of soluble organic foulants in the cake layers analyzed by EEM.

Figure 7. Composition of irreversible (chemically-cleaning) organic foulants analyzed by EEM.

Figure 8. Composition of irreversible (chemically-cleaning) inorganic foulants analyzed by ICP-MS.

Figure 9. Bacterial communities developed in the reactor effluents and membrane foulants. (a) at class level; (b) at genus level.

Table 1. Reactor performances in single-stage and two-stage AFMBRs (Day 30-260).

		Single-stage AFMBR	The 2 nd stage AFMBR in two-stage AFMBR
		Average (min, max)	Average (min, max)
Feed* (mg/L)	pH	7.2 (6.8, 7.6)	7.3 (6.8, 7.8)
	TSS	771 (58, 5900)	257 (37, 1910)
	VSS	464 (20, 2560)	196 (10, 1490)
	TCOD	809 (100, 4590)	411 (36, 3060)
	sCOD	70 (32, 216)	48 (21, 105)
	TOC	25 (12, 97)	17 (4, 72)
Effluent (mg/L)	pH	7.2 (6.8, 7.7)	7.3 (6.9, 8.1)
	TSS	471 (140, 1100)	605 (191, 1670)
	VSS	350 (124, 940)	426 (40, 1310)
	TCOD	556 (64, 2311)	551 (17, 2540)
	sCOD	47 (14, 137)	36 (11, 85)
	TOC	16.3 (7.8, 103.7)	11.2 (4.0, 29.4)
Permeate (mg/L)	pH	7.4 (7.0, 7.8)	7.3 (6.1, 8.0)
	sCOD	24 (4, 53)	18 (5, 56)
	TOC	6.2 (3.2, 11.2)	5.0 (0.8, 11.3)

* The settled sewage was the feed of single-stage AFMBR; The pilot AFBR effluent was the feed of the second-stage AFMBR.

Table 2. Characteristics of cake layer foulants

		Single-stage AFMBR				The 2 nd stage AFMBR in two-stage AFMBR		
Membrane flux (LMH)		5	10	20	30	10	20	30
TSS (mg/m ² day)*		5	192	114	5434	9	184	2262
VSS (mg/m ² day)*		2	56	86	4349	1	119	1612
TOC (mg/m ² day)*		1.0	1.2	1.8	238	0.6	4.6	114
EPS* (mg/m ² day)	Polysaccharides	0.4	0.2	1.6	202	2.1	3.2	281
	Protein	0.9	0.5	1.8	213	0.6	3.1	156
	Poly/Protein	0.44	0.40	0.89	0.95	3.5	1.03	1.80

* n=1 or 2.

Supplementary data:

Table S1. Discharged soluble organic loading* at different conditions

	HRT	Feed soluble organic loading (mg/day)	Flux (LMH)	Discharged soluble organic loading (mg/day)	Rejected soluble organic loading by membrane filtration (mg/day)
Single-stage AFMBR	3 h	1512	5	891	61
			10	767	121
			20	519	243
			30	271	364
2 nd stage AFMBR	2 h	1555	10	976	95
			20	786	190
			30	596	285

* The soluble organic loading is calculated based on the averaged sCOD value.

Table S2. Averaged membrane fouling rate (kPa/day) at different permeate fluxes in single-stage and two-stage AFMBRs.

Flux (LMH)	Single-stage AFMBR	2 nd stage AFMBR
5	0.02	-
10	0.08	0.04
20	0.08	0.12
30	13.31	4.597

Figure S1. Compositions of the soluble organic substances in the effluent of single-stage AFMBR and the 2nd-stage AFMBR of the two-stage AFMBR systems, analyzed by LC-OCD.

Figure S2. Component ratios of the soluble organic substances in the effluent of single-stage AFMBR and the 2nd-stage AFMBR of the two-stage AFMBR systems, analyzed by EEM.

Figure S3. Relationship between accumulated soluble cake layer foulants and membrane fouling rate. It is noted that there was no significant linear relationship of membrane fouling rate with humic acids content ($R^2=0.8323$), building blocks content ($R^2=0.2312$), or LWM acids content ($R^2=0.3126$).