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Novel anaerobic membrane bioreactor (AnMBR) design for wastewater treatment at long HRT and high solid concentration

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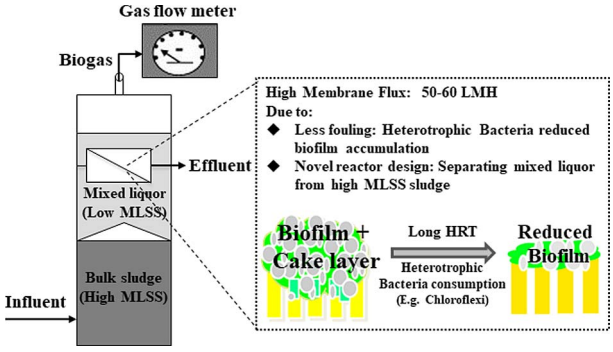
GRAPHICAL ABSTRACT



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ABSTRACT

Performance of two novel designed anaerobic membrane bioreactor (AnMBRs) for wastewater treatment at long hydraulic retention time (HRT, 47 days) and high sludge concentration (22 $g{\cdot}L^{-1}$) was investigated. Results showed steady chemical oxygen demand (COD) removal (> 98%) and mean biogas generation of 0.29 $LCH_4{\cdot}g^{-1}$COD. Average permeates flux of 58.70 $L{\cdot}m^{-2}{\cdot}h^{-1}$ and 54.00 $L{\cdot}m^{-2}{\cdot}h^{-1}$ were achieved for reactors A and B, respectively. On top of reactor configuration, long HRT caused biofilm reduction by heterotrophic bacteria Chloroflexi resulting in high membrane flux. Mean total membrane resistances ($2.23 \times 10^9\ m^{-1}$) and fouling rates ($4.00 \times 10^8\ m^{-1}{\cdot}day^{-1}$) of both reactors were low suggesting better membrane fouling control ability of both AnMBRs. Effluent quality analysis showed the effluent soluble microbial products (SMP) were dominated by proteins compared to carbohydrates, and specific ultraviolet absorbance (SUVA) analysis revealed effluent from both reactors had low aromaticity with SUVA < 1 ($L{\cdot}mg^{-1}{\cdot}m^{-1}$) except for the first ten days.

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1. Introduction

Anaerobic membrane bioreactor (AnMBR) is drawing much attention for its potential to treat domestic, municipal and industrial wastewaters due to (i) its versatility separating hydraulic retention time (HRT) and solid retention time (SRT) thereby allowing the slow growth of anaerobic bacteria, (ii) having a small footprint, (iii) maintaining higher biomass concentration, (iv) providing higher treatment capacity and (v) producing effluent with excellent quality (Kunacheva et al., 2017b; Stuckey, 2012). Moreover, AnMBR can reduce the major problems of aerobic membrane reactors such as high energy consumption for aeration, high solid yield, emission of carbon dioxide and large footprints by integrating unit operations (Stuckey, 2012). However, the major problem of AnMBR is the rapid decline of the permeate flux over operation time or increase in transmembrane pressure as a result of membrane fouling (Guo et al., 2012; Tang et al., 2010).

Mixed liquor suspended solid (MLSS) concentration in membrane bioreactor systems is believed to cause membrane fouling. Park et al. (2015) studied the process of particle deposition (caking) and sloughing-off from membrane surface continuously during MBR operation could lead to the cessation of accumulation of solids at some point indicating that high and increasing MLSS concentration will not increase development of cake layer proportionally. There are contradictory reports about the impact of MLSS concentration on membrane fouling. Judd (2010) reported, high MLSS concentration can affect MBR performance causing decrease in the ratio of mixed liquor volatile suspended solid (MLVSS) to MLSS (MLVSS/MLSS) due to the accumulation of inert compounds and this in turn resulted in the accumulation of solids in the membrane channel. Likewise, the membrane resistance was found to increase exponentially as a function of MLSS concentration (Meng et al., 2007). Contrary to those findings increase in MLSS concentration showed no significant change in fouling but observed significant increase in critical flux value (Le-Clech et al., 2003). Despite those contradictory reports by the impact of MLSS on membrane fouling, there was a report on the importance of critical MLSS concentration (10.50 $g{\cdot}L^{-1}$), above which the sludge particles could be kept in the sludge resulting in better filterability (Lousada-Ferreira et al., 2015).

Apart from MLSS, extracellular polysaccharide substances (EPS) and soluble microbial product (SMP) play critical role in membrane fouling by decreasing flux and increasing trans-membrane pressure (Guo et al., 2012; Herrera-Robledo et al., 2011). SMPs are released into solution from substrate metabolism and biomass decay of which 20% is from soluble portion of EPS (Kunacheva and Stuckey, 2014). Besides, Ni et al. (2011) reviewed classification of SMP into: biomass associated products (BAP) and utilization associated products (UAP), where the former is the type of SMP that is produced from the hydrolysis of biomass, in particular from EPS while the latter is produced directly as part of electron-donor oxidation.

Concentrations of SMP and EPS build up inside the reactor with increasing in HRT which in turn causes membrane fouling. Fouling due to the accumulated SMP can follow different mechanisms, i.e. accumulation inside the pores by causing standard, intermediate, complete blocking as well as cake layer formation (Herrera-Robledo et al., 2011). BAP plays significant role in MBR fouling as longer SRT might lead to clogging of membrane pores and/or deposition (Ni et al., 2011). Tang et al. (2010) reported dissolved organic matter which mainly contained carbohydrate, proteins and more biologically recalcitrant compounds like fulvic and humic acids, accounting for 26–57% membrane fouling in wastewater treatment systems. Similarly, EPS contributes 50–80% of total organic matter in biofilm of the fouled membrane (Guo et al., 2012). On the other hand, SMP plays greater role in increasing chemical oxygen demand (COD) of the effluent from anaerobic wastewater treatments facilities and reported to contain phthalates (up to 3 $mg{\cdot}L^{-1}$) and other long chain alkane and alkenes (Kunacheva and Stuckey, 2014). It is reported that influent COD contributes only 2% of COD in the effluent SMP under optimum operating conditions and can increase up to 17% under abnormal operating conditions, such as nutrient limitation, presence of toxicity and variable substrate composition (Kunacheva and Stuckey, 2014). Similarly, biomass associated SMP which is mainly controlled by EPS concentration can approach 4–5% of the influent substrate as COD for moderate to high SRT. Unlike biomass associated SMP, the concentration of utilization associated SMP in the effluent is high only for relatively small SRT since their degradation rate is high due to increased accumulation of biomass at high SRT (Ni et al., 2011).

For most optimal AnMBR operations, HRT ranges from 4 h (Salazar-Peláez et al., 2011) to 2 days (Baek et al., 2010) while the MLSS concentration ranges between 8 and 12 $g{\cdot}L^{-1}$. However, under circumstances when the organic matter is not readily biodegradable, applying longer HRT could alleviate the problem though it requires large foot print. Qiao et al. (2013) studied HRT between 30 and 70 days to study thermophilic anaerobic digestion of coffee grounds with and without waste activated sludge as co-substrate using a submerged AnMBR, which justifies the importance of long HRT for some categories of wastes. Several studies dealt with the impact of HRT on AnMBR performance; however, the AnMBR performance under harsh conditions of high sludge MLSS concentration ($\sim$22.00 $g{\cdot}L^{-1}$) coupled with long HRT (47 days) was not reported. These harsh environments are challenging for AnMBR operation and maintenance in view of membrane fouling and effluent quality. This work investigated the applicability of novel AnMBR design where the introduction of a three phase separator between bulk sludge and membrane module could mitigate membrane fouling by enhancing solid stratification. Introduction of a three phase separator was meant to add structural advantage to mitigate membrane fouling by minimizing scouring of bulk sludge that reaches membrane module which otherwise will contribute to membrane fouling. The AnMBR used in this work was a semi-pilot scale, and to the best of our knowledge this design is studied for the first time in an AnMBR apparatus (We have applied a patent prior to submission of this work, Chinese patent number: 201516667001.7). Hence, this study aimed at assessing the impact of high sludge MLSS concentration coupled with long HRT on the performance of novel AnMBR design for wastewater treatment focusing on membrane fouling and effluent quality.

2. Materials and methods

2.1. Reactor set-up and operation

Two replicates of submerged AnMBRs were operated in semi-continuous mode (Fig. 1). The reactors consisted of sludge digestion unit, and flat sheet membrane polyvinylidene fluoride (PVDF) module (Dafu membrane Co. Ltd, China) with pore size of 0.40 μm and total filtration area of 0.14 m^2 submerged in the mixed liquor above three phase separator (Fig. 1). The working volumes of the reactors were 94 L and both reactors were covered by heating coat to maintain the temperature in mesophilic range, 37 ± 1 °C. The hydraulic retention time (HRT) was maintained at 47 days to induce harsh operational conditions while the sludge retention time (SRT) was very large as the sludge was not wasted intentionally. Membrane filtration was operated with intermittent back washing mode (30 min filtration followed by 30 s back washing) to control fouling. The back washing procedure followed reversing the direction of flow with the same flux as operating flux. The trans-membrane pressure was monitored using vacuum pressure gauge and no chemical cleaning was applied throughout the experimental duration.

2.2. Feed wastewater and inoculum

Synthetic wastewater with following composition was used: D-Glucose (30.96 g.), sodium acetate (19.23 g), ammonium chloride (4.30 g), dipotassium phosphate (1.60 g), yeast extract (1.00 g)

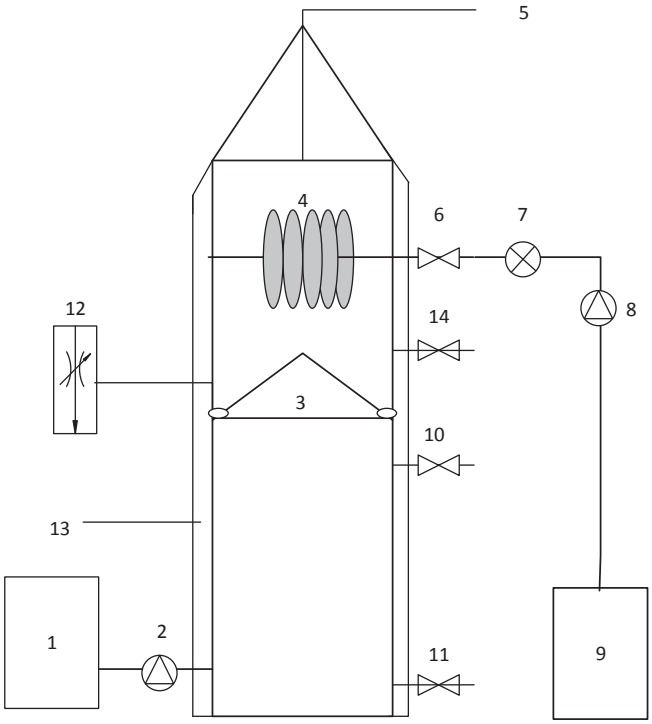

Fig. 1. Schematic diagram of the submerged AnMBR: (1) Feed tank, (2 & 8) Peristaltic tank, (3) Three-phase separator, (4) Membrane module, (5) Biogas outlet, (6, 10, 11 & 14) Gate valve, (7) Pressure gauge, (9) Effluent tank, (12) Temperature control unit and (13) Heating cover.

dissolved in a liter of deionized water and all analytical grade reagents (Xu et al., 2011). Trace elements added were mentioned elsewhere (Adav and Lee, 2008). The synthetic wastewater was fed to the reactor using peristaltic pump with inflow rate of 2 $L{\cdot}d^{-1}$. The seed sludge was collected from Tuan Dao municipal wastewater treatment plant in Qingdao, China. 22.00 $g{\cdot}L^{-1}$ of MLSS and 13.00 $g{\cdot}L^{-1}$ of MLVSS were initial solid concentrations.

2.3. Sludge and effluent characterization

2.3.1. MLSS, MLVSS and EPS extraction and analysis

Duplicate samples of sludge were collected from bulk (bottom of the reactor) and mixed liquor (near membrane module) for sludge characterization. The MLSS and MLVSS concentration of the samples were analyzed according to standard Methods (1999). EPS from the bulk sludge and mixed liquor were extracted according to the procedure indicated elsewhere (Adav and Lee, 2008). EPS extraction followed the route: ultrasound–formaldehyde–NaOH from the methods mentioned in literature (Adav and Lee, 2008).

2.3.2. Specific methanogenic activity (SMA) analysis

Specific methanogenic activity assay was conducted using serum bottle technique at three stages (day 0, 25–30 and 60–70) of the study. Samples were collected in hermetically sealed reactors of 350 mL with a working volume of 200 mL. The inoculum to substrate (sodium acetate) ratio was fixed at 2:1 (VSS/COD, Angelidaki et al., 2009). Reactors were filled with 200 mL mixture of substrate, inoculum and nutrient solution under the gas mixture of nitrogen and carbon dioxide (80:20) for two minutes to ensure anaerobic condition (Ho and Sung, 2010). The final mixture was incubated at 35 °C in water bath. Volume of methane gas produced was recorded every 12 h after the gas mixture was passed through 2% NaOH to remove carbon dioxide (Esposito et al., 2012). The SMA value was calculated from the slope of the plot of cumulative production of methane gas versus time. The slope was determined from the most linear portion of the curve, and the slope was divided by MLVSS concentration to obtain the SMA value.

2.3.3. SMP extraction and analysis

SMP analysis was carried out using filtered effluent and mixed liquor through membrane with pore size of 0.45 μm. The filtered samples were stored at 4 °C prior to analysis. The filtrate was used for measurement of total carbohydrate and total protein by Dubios phenol-sulfuric acid method with glucose as the standard and the Lowry method with bovine serum albumin as the protein standard, respectively (DuBois et al., 1956; Lowry et al., 1951).

2.3.4. Specific ultraviolet absorbance

Specific ultraviolet absorbance represents the level of aromaticity in the effluent was analyzed according to USEPA (Potter and Wimsatt, 2005). SUVA was determined from the ratio of effluent ultraviolet absorbance (UVA) at a wave length of 254 nm and dissolved organic carbon concentration (Meng et al., 2017). Moreover, dissolved organic carbon concentration in the effluent samples were analyzed using LiquiTOC II elementar analyzer (Elementar LiquiTOC, Elementar Co., Hanau, Germany).

2.4. Fouling analysis

2.4.1. Total resistance and fouling rate calculation

The total membrane resistance was calculated applying Darcy's law according to Eq. (1):

$$R_t = \frac{TMP}{\mu J} \tag{1}$$

where J is the membranes permeate flux ($m^3{\cdot}m^{-2}{\cdot}s^{-1}$), TMP is transmembrane pressure (Pa), μ is dynamic viscosity (Pa·s) of water, and R_t is the total membrane filtration resistance (m^{-1}). On the other hand fouling rate was calculated by Eq. (2) (Xue et al., 2016):

$$\Delta R = \frac{R_{t1} - R_{t0}}{\Delta t} \tag{2}$$

where Δt is filtration time (h) between initial (R_{to}) and final (R_{t1}) membrane filtration resistances.

2.4.2. Foulant layer characterization

Scanning electron microscopy (SEM) technique was used to qualitatively characterize foulant layer on fouled membrane surface. Pristine and fouled membrane samples were prepared before SEM imaging by coating with a thin layer of gold in order to reduce membrane surface charge. SEM images of the membrane surface were taken at voltage of 5 kV using the Hitachi S-4800, Japan. FTIR analysis was carried out to identify the major functional groups on pristine and fouled membrane surfaces (Thermo Scientific™ Nicolet™ iN™10, China).

2.5. DNA extraction and high through put sequencing

Understanding the dynamics of microbial community structure in activated sludge systems helps to predict the effect of the changes in different operational parameters on microbial structure of activated sludge systems. Microbial community dynamics across the operational period were analyzed following DNA extraction and high through put sequencing techniques. Sludge samples were collected and stored at −20 °C before DNA extraction and analysis. DNA extraction was carried following manufacturer's manual using PowerSoil® DNA Isolation Kit from Mo-Bio Laboratories, Inc. (Australia). Extracted DNA was eluted to a final volume of 100 mL and stored at −20 °C before further analysis. Following extraction DNA samples were sent to a commercial laboratory (BGI, China) for high-throughput pyrosequencing (Illumina MiseqnPE250). 515F (50-GTG CCA GCM GCC GCG GTA A-30) and 806R (50-GGA CTA CHV GGG TWT CTA AT-30) primers were amplified

by PCR targeting V4–V5 region of the microbial 16S ribosomal RNA gene to simultaneously obtain bacterial and archaeal information, respectively (Lü et al., 2016). Sequences of the samples were deposited into the NCBI Sequence Read Archive (SRA) database (PRJNA388574).

2.6. Analytical methods

Molybdenum blue and indophenol blue methods were used to analyze total Kjeldahl nitrogen (TKN) and total phosphorous (TP), respectively (APHA, 2005). Likewise, chemical oxygen demand (COD) was measured according to standard methods (APHA, 2005). COD concentrations in permeate of the AnMBRs were directly measured without filtration. Composition of biogas was measured by gas chromatograph (SP 6890, Shandong Lunan Inc., China). The gas chromatograph was equipped with a Porapak Q stainless steel column (180 cm long, 3 mm outer diameter) and a thermal conductivity detector. The temperatures of the injector, detector, and oven were 50, 100 and 100 °C, respectively and argon was used as carrier gas.

3. Results and discussion

3.1. Carbon metabolism and effluent quality

3.1.1. COD removal and effluent specific ultraviolet absorbance

The COD removal profiles of AnMBRs run in parallel for 70 days were presented in Fig. 2a. Carbon metabolism of both reactors showed high performance with mean COD removal of 98.84% and 98.75% for reactor-A and reactor-B respectively. The COD removal of both reactors was stable during the operational period except in the first ten days where it was down to 97.00%. The decreased COD (97.00%) removal could be due to acidification in the respective reactors as it was startup period in the first ten days but the pH was still in the optimum range of methanogenesis (data is not shown). This result agrees with previous study where more than 90% COD removal at MLSS concentration between 20 and 25 $g{\cdot}L^{-1}$ was achieved (Fuchs et al., 2003).

SUVA is commonly used to analyze the aromatic content of water samples (Her et al., 2003). The total organic carbon (data not shown) values of the AnMBR effluent samples ranged between 40 and 140 $mg{\cdot}L^{-1}$ over the operation period. Moreover SUVA values for effluent samples from both reactors were less than 1.00 except at the start-up period, which might be due to scouring of sludge that could result in dissolution of humic matter which ends-up in the effluent as shown in Fig. 2b. The mean SUVA values were 0.63 and 0.61 $L{\cdot}mg^{-1}{\cdot}m^{-1}$ for reactor A and B respectively. Her et al. (2003) reported that SUVA value of 7.49 $L{\cdot}mg^{-1}{\cdot}m^{-1}$ corresponded to high aromaticity. In both AnMBRs, most of the SUVA values were < 1.00 which indicates that the effluent samples from this study had very low aromaticity.

3.1.2. SMP, total EPS, MLSS and MLVSS profiles

Concentrations of SMP proteins in effluents from both reactors were found to be greater than corresponding carbohydrates, especially during the first twenty (0–20) and last fifteen days (55–70) as indicated in Fig. 3a. The discrepancies between the carbohydrate and protein could be due to multiple factors, one of which might be accumulation/production is directly related to increasing HRT that resulted in biomass decay (Barker and Stuckey, 1999). However, it is reported that accumulation of SMP and metabolic products is due to the rejection by membrane and it improved effluent quality (Shin and Kang, 2003). Even though significant amount of protein is rejected by the membrane and biofilm layer that resulted in high concentration of mixed liquor protein, there is high concentration of SMP protein in the effluent (this work). Contrary to high concentration of SMP protein, SMP carbohydrate in the effluent was found to be very low and it could be due to degradation by biomass since carbohydrates are readily biodegradable by microbes compared to proteins.

SMP: VSS between 0.04 and 0.05 for submerged anaerobic membrane bioreactor was considered as an indication of biomass decay due to sludge ageing (Aquino et al., 2006). The SMP: VSS of effluent with respect to bulk sludge was 0.0022 and 0.0020 for reactor A and B respectively. Similarly, SMP: VSS of effluent with respect to mixed liquor above the three phase separator was found to be 0.020 and 0.010 for reactor A and B respectively. The discrepancy between the two ratios justifies the accumulation of more SMP in the mixed liquor compared to bulk sludge and the higher SMP concentration in the mixed liquor must be released from the bulk sludge emanating from biomass decay induced by long HRT. Furthermore, Kunacheva et al. (2017a) reported that dynamic membrane developed on the membrane surface played significant role in retaining/rejecting low molecular weight compounds, while permeating high molecular weight compounds which could be another reason for higher concentration of SMP protein in the effluent irrespective high fouling, and this might be the same scenario for this work.

The MLSS concentrations under the three phase separator in both reactors increased very slightly at the end of the experiment, i.e., from initial 22.30 $g{\cdot}L^{-1}$ to 26.60 $g{\cdot}L^{-1}$and 25.80 $g{\cdot}L^{-1}$for reactors A and B

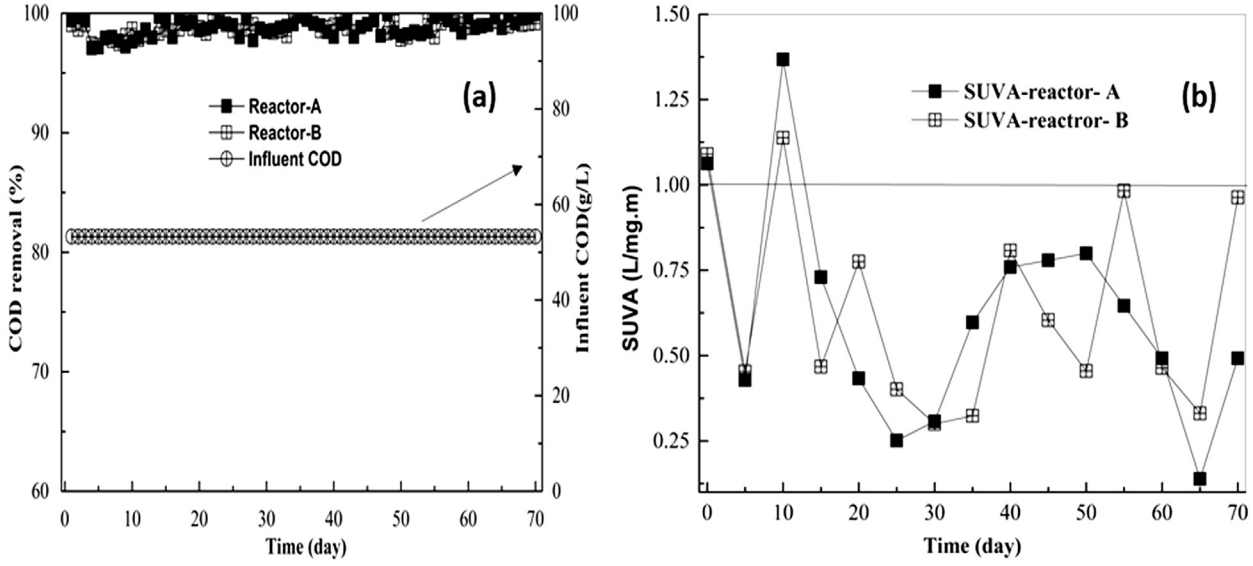

Fig. 2. Carbon metabolism and effluent quality (a) Influent COD and COD removal (b) Specific UV absorbance of effluent sample.

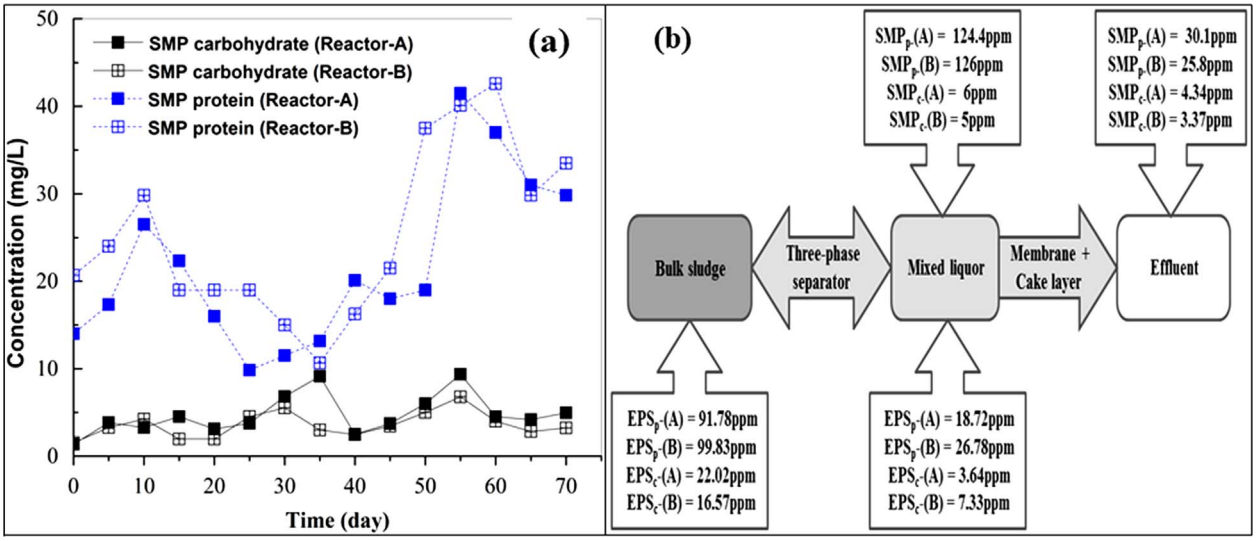

Fig. 3. Effluent and biomass properties (a) SMP profile at different stages of the operation (b) Summary of mean EPS and SMP composition over the operational time.

respectively. Similarly the MLVSS values showed increment from the initial value of (13.10 g·L^{-1}) at the start of the experiment to 15.70 g·L^{-1} and 14.90 g·L^{-1} for reactors A and B, respectively. The ratio of MLVSS/MLSS at the start of the experiment was 0.59 and these ratios almost remain unchanged at the end of the operation, where it was 0.59 and 0.58 for reactors A and B, respectively. The mean values of MLSS concentrations above three phase separator (where the membrane was submerged) were 3.78 g·L^{-1} and 6.23 g·L^{-1} for reactor A and B respectively. Moreover the biomass concentrations (MLVSS) for both reactors above three phase separator were 2.16 g·L^{-1} and 3.69 g·L^{-1} resulting in MLVSS/MLSS ratio of 0.57 and 0.59 for reactor A and B respectively. This clearly shows introduction of three phase separator below membrane module enhanced stratification of solid between the bulk sludge assisted by the inherent stratification along depth of the reactor.

On the other hand Luna et al. (2014) reported EPS stratification along depth of a reactor is affected by total suspended solid stratification where highest EPS production was expected at higher part of the reactor compared to bottom and middle parts. Contrary to this report the finding of this work showed highest total EPS production from bottom of the reactor (bulk sludge) as opposed to amount of total EPS in the mixed liquor above the three phase separator for both reactors. Moreover, composition of EPS (as protein and carbohydrate) was found to be dominated by protein throughout the operation period (Fig. 3b). The later could be due to the hydrophobicity of the sludge as previously reported in literature (Jorand et al., 1998). Moreover, Hu et al. (2017) reported significantly high concentration of EPS protein compared to EPS polysaccharide from pilot scale anaerobic membrane bioreactor for treating tetrahydrofuran pharmaceutical wastewater at different HRTs. Even though the overall total EPS was found to be dominated by protein, its percentage decreased with operation time and hence decreased hydrophobicity, which may be associated to the increase in the carbohydrate percentage as shown in Fig. 3b. However, the SMP protein concentration in the mixed liquor is found to be high compared to total EPS protein form bulk sludge. The later phenomena could be due to abundance of smaller flocs in the mixed liquor with large surface area that release SMP. Another factor could be the harsh environment on the upper part of the reactor with lower availability of substrate, which resulted in release of EPS (Lin et al., 2011).

3.1.3. TKN and TP

During operation of anaerobic processes removal of nutrients was limited to utilization for biomass growth, which is not high enough for nutrient removal (Smith et al., 2012). As a result of this inherent limitation of anaerobic process in general and AnMBR in particular, there is a clear trend of increase in concentrations of TKN and TP in effluent samples from both reactors over operation time as presented in Supporting information. Another important contributor for increased effluent TKN and TP could be long HRT since long HRT (47 days) may result in biomass starvation and decay, which would have resulted in the release of nutrients to the effluent. Hence, the effluent samples from this work contained high nutrient concentration which dictates the need for post-treatment using method (s): partial nitritation/nitrification coupled with anammox, and physical/chemical nutrient removal methods, such as phosphorous removal by flocculation/coagulation, zeolite adsorption for ammonium removal, and ion exchange resins specifically designed for ammonium and phosphate removal (Smith et al., 2012).

3.2. Membrane performance and fouling analysis

3.2.1. Transmembrane pressure (TMP) and permeate flux

The TMP for the first six days were zero due to its operation under gravity filtration as presented in Fig. 4a. Following gravity filtration the TMP increased sharply to about 40 kPa was due to standard and intermediate pore blocking mechanisms. This phenomenon agrees with previous reports that membrane fouling was a three stage process where the initial stage is rapid fouling caused by initial pore blocking and adsorption of solutes, which resulted in increased *trans*-membrane pressure (Drews, 2010; Kim et al., 2011). Afterwards, the TMP decreased between days 12 and 22 to 30 kPa following development of stable cake layer. The TMP increased sharply from day 22 to day 35 from about 30 kPa for both reactors to between 40 and 45 kPa. The TMP increment between days 22 and 35 could be due to pore blocking and development of dense cake layer resulting in cake filtration. Operating flux was decreased following sharp increase in TMP from day 22 to day 35 to maintain sustainable flux without chemical cleaning.

Even though the operating flux was decreased for both reactors after day 55, the TMP for reactor B was found to be higher than that of reactor A which could be due to higher fouling in reactor B. The higher membrane fouling in reactor B could be due to high concentration of SMP protein compared to reactor A (Fig. 3a). Ozgun et al. (2015) investigated higher SMP concentration was one of the contributing factors for membrane fouling during treatment of municipal wastewater treatment using up flow anaerobic sludge blanket reactor combined with ultrafiltration membrane. On the other hand, hydrophobicity is exacerbated by high protein concentration which is another factor for membrane fouling (Arabi and Nakhla, 2008). The latter could be more

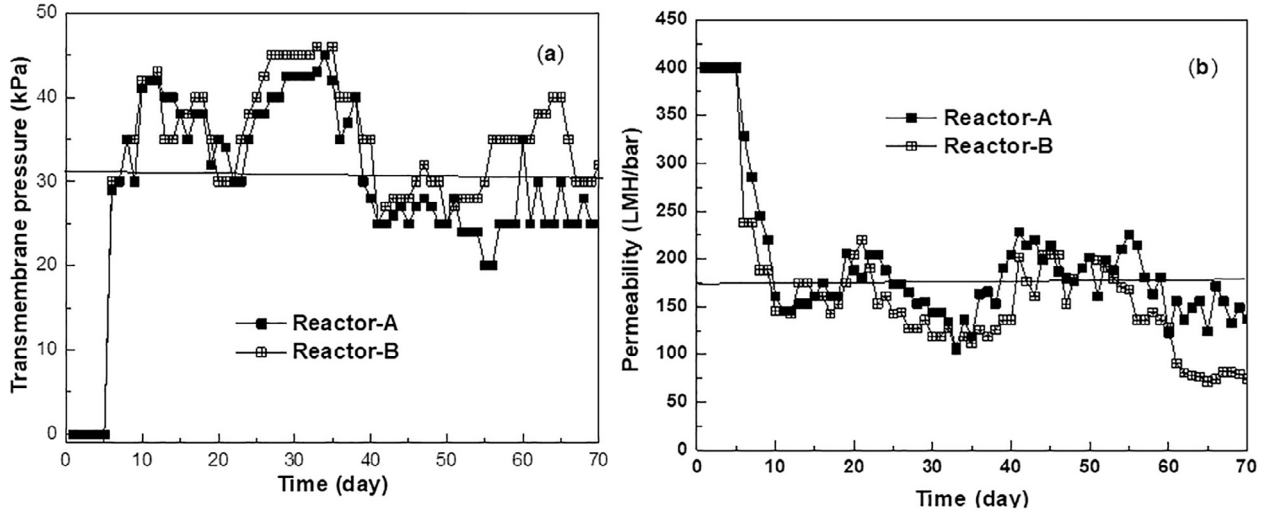

Fig. 4. Transmembrane profile (a) and membrane permeability (b) across operation period.

for reactor B due to more SMP protein compared to reactor A. Furthermore high MLSS concentration is expected to induce increased production of EPS and SMP that contribute to irreversible fouling (Park et al., 2015), this strengthen the argument that high SMP production is related to high MLSS concentration which in turn caused deterioration of membrane performance.

3.2.2. Membrane fouling analysis and characterization

3.2.2.1. Fouling rate and total resistance. Day 6, 33, 38 and 70 (Table 1) were selected for analysis of fouling rate and total resistance due to TMP jump at these stages (Fig. 4a). The total resistances and corresponding fouling rates during the operation period were calculated according to Eqs. (1) and (2). The fouling rate on day 6 was higher for both AnMBRs (1.10×10^9 and $1.50 \times 10^9\ m^{-1}{\cdot}day^{-1}$ for A and B respectively), which might be attributed to rapid fouling caused by initial pore blocking and adsorption of solutes following transition from gravity filtration to vacuum filtration as shown in Table 1 (Drews, 2010; Kim et al., 2011). On the subsequent stages (day 6–33), the fouling rate decreased (6.80×10^8 and $5.90 \times 10^8\ m^{-1}{\cdot}day^{-1}$ for AnMBR A and B, respectively) while TMP increased resulting in maximum values of total resistances (3.36×10^9 and $3.43 \times 10^9\ m^{-1}$ for AnMBRs A and B, respectively), which might be due to the development of dense cake layer. The sharp increase in resistance values were controlled by decreasing the flux, so those resistances were found to decrease after day 33 (Table 1). Even though the operating flux were decreased to control fouling rate, total membrane resistance were found to increase at the later stage of this experiment (Table 1), which might be due to the development of dense cake layer as presented in Supporting information.

3.2.2.2. SEM and FTIR analysis of membrane samples. The surface and cross section morphology of pristine and fouled membranes were presented in Supporting information. The SEM image showed that fouled membrane surface changed due to foulant matrix attached by developing cake layer. Development of this cake layer could explain the TMP jump that occurred at different stages of this experiment as indicated in Fig. 4a. Composition of cake layer is attributed to deposition/attachment of macromolecules such as protein, carbohydrate and phospholipids as confirmed from FTIR scanning. Moreover, the foulants particles and/or soluble microbial products are logged inside the membrane channels which might result in pore narrowing as shown in Supporting information.

On the other hand, major functional groups on pristine and fouled membrane surfaces are indicated on FTIR spectra as shown in Supporting information. The FTIR spectrum shows broad band at 3406.64 cm^{-1} and 3289.92 cm^{-1} for pristine and fouled membrane samples respectively. These could be due to O—H stretching of hydroxyl functional groups (Rao et al., 2006). A peak with 2934 cm^{-1} on fouled membrane might be due to asymmetric stretching vibration of CH_2 (Guibaud et al., 2003), which was present on pristine membrane Moreover, there were closely related peaks that appeared at 1667 and 1639 cm^{-1}, 1174.7 cm^{-1} and 1277 and 1234 cm^{-1} for pristine and fouled membranes, respectively, which could be related to protein secondary structures of amides I (C—O stretching), II (N—H in plane) and III (C—N stretching) respectively (Sun et al., 2016). The signal detected at 1072 cm^{-1} belongs to C—O bonds which might be alcohols, ethers and polysaccharides (Jouraiphy et al., 2008). Furthermore, the bands that appeared in the finger print region; mainly at 1277–1234 cm^{-1} and less than 1000 cm^{-1} were related to phosphate esters and nucleic acids as previously reported (Jouraiphy et al., 2008). Thus, it is evident to conclude the cake layer on the membrane surface is composed of macromolecules such as proteins, polysaccharides and phosphate esters and/or nucleic acids.

3.3. Sludge characterization

3.3.1. Specific methanogenic activity (SMA) and methane production

Specific methanogenic activity of inoculum was found to be

Table 1
Total resistance and fouling rates over operational period.

Sampling time from the commencement (d)	HRT (d)	Total resistance (m^{-1})		Fouling rate ($m^{-1}{\cdot}day^{-1}$)	
		Reactor-A	Reactor-B	Reactor-A	Reactor-B
6	47	1.10×10^9	1.51×10^9	1.10×10^9	1.50×10^9
33	47	3.36×10^9	3.43×10^9	6.80×10^8	5.90×10^8
38	47	1.68×10^9	2.04×10^9	1.10×10^8	2.60×10^8
70	47	2.63×10^9	4.84×10^9	2.10×10^8	3.00×10^8

0.35 LCH_4-COD·$gVSS^{-1}$·d^{-1} for acetate. This result shows the biological activity of the inoculum agrees with minimum criterion for SMA which is 0.14 LCH_4-COD·$gVSS^{-1}$·d^{-1} for sludge (Angelidaki et al., 2009). Similarly, SMA value for both AnMBRs on day 30 was found to be 0.33 and 0.32 LCH_4-COD·$gVSS^{-1}$·d^{-1} for reactor A and B, respectively. Furthermore, the SMA on day 60 showed slight decline to 0.33 and 0.28 LCH_4-COD·$gVSS^{-1}$·d^{-1} for reactor A and B, respectively. Based on the SMA values, it is safe to say that the sludge had good biological activity even though SMA values showed slight decline in the subsequent stages of the experiment for reactor-B. Similarly, mean specific methane productions of both reactors over the operation period were 0.29 and 0.28 LCH_4·g^{-1}COD removed for reactor-A and reactor-B, respectively. This agrees with the previous report of mean specific methane production of 0.29 LCH_4·g^{-1}COD for treatment of low to high strength wastewaters using anaerobic biofilm membrane bioreactor (Li et al., 2017). Moreover, the average methane concentrations (percentage) in biogas over operation period of this work were 62.70% and 61.83% for reactors A and B, respectively as presented in Supporting information.

3.3.2. Microbial diversity analysis

Genus level microbial community analysis of inoculum and bulk sludge samples collected on day 45 revealed that, *Methanolinea* (60%, 63% and 36%) were the most abundant archaeal communities followed by *Methanosaeta* (37%, 32% and 63%) for reactor-A, reactor-B and inoculum respectively (Fig. 5a). It is been reported that the filamentous acetoclastic archaea (*Methanosaeta)* has the ability of initiating granulation, which could be one of the reasons for stable operation at such high MLSS as 22 g·L^{-1} (Angenent et al., 2004). *Methanoculleus* were the third abundant genera of archaeal group contributing 1.85%, 3.60% and 0.39% in AnMBR-A, AnMBR -B and inoculum, respectively.

High-throughput sequencing of phylum revealed, *Euryarchaeota* was found to be joint largest abundant phyla of archaea with *Firmicutes* accounting for 19%, 19% and 6% in reactor-A, reactor-B and inoculum, respectively (Fig. 5b). The abundance of *Euryarchaeota,* methanogenic archaea increased in both reactors with operation time which could be due to accumulation of chloride ions coming from feed wastewater as a result of long HRT. The second abundant phylum was *OP1* with 13%, 12% and 6% in the above respective order. *Proteobacteria* was the third abundant phyla detected accounting for 9%, 8% and 18% respectively in reactor-A, reactor-B and inoculum. According to Watanabe et al. (Watanabe et al., 2016), *Proteobacteria* is believed to cause membrane fouling and form biofilm on membrane surface. But, result of this study showed percentage of *Proteobacteria* decreased by 50% in both reactors compared to inoculum, which might be one of the reasons for less fouling in this work compared to previous works, as can be inferred from the total resistance and fouling rate values (Table 1). *Bacteroidetes* was the third abundant phyla in the order of 9%, 7% and 20% AnMBR-A, AnMBR -B and inoculum, respectively. *Thermotogae* was fifth abundant bacterial phyla with 6%, 9% and 12% respectively in reactor-A, reactor-B and inoculum. *Thermotogae* appeared in all sludge samples probably because the inoculum was collected from wastewater treatment plant operating in thermophilic condition. However, its abundance decreased in both reactor A and B compared to inoculum due to the change in operational temperature from thermophilic to mesophilic. *Chloroflexi* was the sixth abundant phyla accounting for 4%, 4% and 9% respectively in reactor-A, reactor-B and inoculum. Okabe et al. (2005) reported that the heterotrophic bacteria *Chloroflexi* prefers consuming biomass associated soluble microbial products in the biofilm thereby minimizing accumulation of organic waste which contributes to the control of membrane.

On the other hand, Shannon and Simpson indices were used to evaluate the richness and evenness of species in the community. The analysis result revealed that Shannon index of seed sludge was higher than the replicate reactors suggesting the decrease in microbial richness with operational time which might be due to the harsh operating conditions (Table 2). Simpson diversity index of the seed sludge was lower than that of both AnMBRs, which also suggested reduced microbial diversity because of the AnMBR operation.

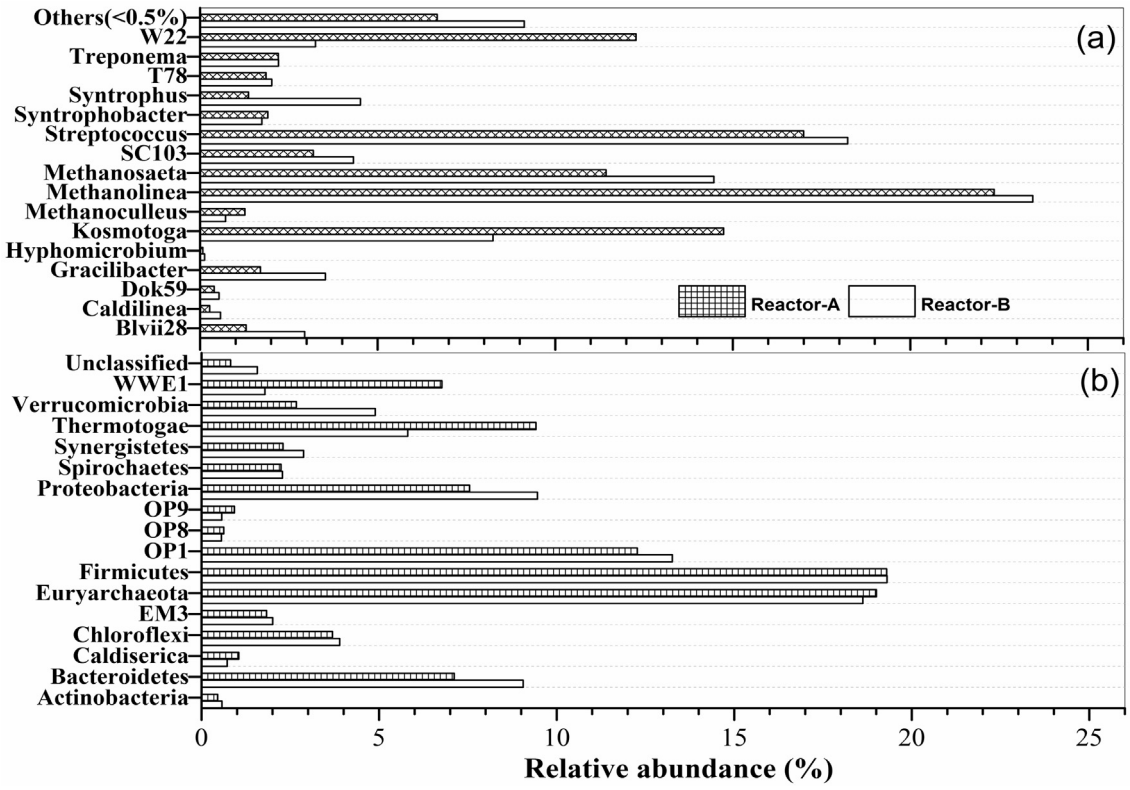

Fig. 5. Relative abundance of the microbial diversity in genus (a) and phylum (b) levels.

Table 2
Alpha diversity indices of bacterial clone libraries.

Sample name	OTU number	Shannon	Simpson
Reactor-A	710	3.97	0.05
Reactor-B	644	3.77	0.05
Seed	913	4.92	0.02

4. Conclusion

The impact of high MLSS concentration ($\sim 22.00\ g{\cdot}L^{-1}$) coupled with long HRT (47 days) on AnMBR performance in terms of membrane fouling and effluent quality was investigated. Satisfactory effluent quality in terms of COD removal > 98% and SUVA < 1.00; however, effluent SMP proteins were higher than the corresponding carbohydrates at all stages of this study. Due to the consumption of biofilm by heterotrophic bacteria *Chloroflexi* and the novel reactor design, mitigated membrane fouling with high average specific fluxes of 58.70 and 54.00 LMH for reactors A and B were achieved, respectively.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.11.025.

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