Powdered activated carbon addition for fouling control in anaerobic membrane bioreactor

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Abstract

The effect of powdered activated carbon (PAC) addition to a conventional anaerobic membrane bioreactor (AnMBR) on removal performance and membrane fouling was explored in this study. The optimal one-off PAC dose could increase average chemical oxygen demand (COD) and total organic carbon (TOC) removal rates up to 15.7% and 15.6%, respectively. The PAC addition exhibited not only lower TMP increase rate, but also reduced soluble microbial product (SMP) and extracellular polymeric substances (EPS). Higher protein to polysaccharide ratio of SMP and higher zeta potential in AnMBR with PAC could enhance hydrophobicity and flocculation ability, thereby effectively alleviating membrane fouling. Lower total membrane resistance and pore blocking resistance indicated that PAC addition could prevent both severe pore blocking and irreversible fouling, due to the lower polysaccharide level in SMP of the cake layer. Moreover, PAC addition facilitated the decrease in fouling-related bacteria such as *Cloacibacterium* and *Paludibacter*.

Keywords: Anaerobic membrane bioreactor; Membrane fouling; Powdered activated carbon; Microbial community; Fouling-related bacteria

1. Introduction

As water scarcity has been a huge threat worldwide due to the fast population growth and the resulting increased demand for fresh water, wastewater treatment and reuse have become attractive options as an alternative water resource. Anaerobic membrane bioreactor (AnMBR), an integration of anaerobic digestion and membrane filtration technology, has gained great popularity, because of the benefits over aerobic membrane bioreactor (MBR) like low sludge generation and potential energy usage. However, one of the biggest barriers is membrane fouling, which is due to the interaction between the membrane itself and the components from the sludge suspension, which causes a decrease in operation productivity, periodic membrane cleaning and replacement, and the reduction of membrane lifespan. It is reported that the microbial metabolites such as soluble microbial product (SMP) and extracellular polymeric substances (EPS) are the major determinants of biofouling, and their components such as proteins and polysaccharides are normally considered as major contributors to membrane fouling (Guo et al., 2012).

The occurrence of membrane fouling in both aerobic and anaerobic MBRs is generally characterised by initial pore blocking and subsequent cake layer formation. However, the characteristics of membrane fouling in AnMBR can be significantly different to that in aerobic MBR due to the difference of sludge characteristics (Lin et al., 2013). While the cake layer formed in aerobic MBR was reversible, the cake layer in AnMBR was irremovable by relaxation or back flushing due to consolidation (Di Bella et al., 2007; Jeison and van Lier, 2007). Moreover, the cake layer thickness was 1900–2100 μm in AnMBR, which was much higher than that of 20–200 μm in aerobic MBR. In addition, the operating temperature could also significantly affect membrane fouling in AnMBR. Higher filtration resistance was observed when submerged AnMBR was operated under 55 ℃, compared to the operation

under 37 ℃, as high temperature could lead to smaller floc size as well as more production of SMP and EPS (Charfi et al., 2012; Lin et al., 2009).

As a fouling control strategy for membrane bioreactors, pretreatment of feed water, such as acid, alkaline, ozone pretreatment, and the addition of fouling reduction enhancers, have been widely applied in many former studies. The addition of adsorbents and flocculants as fouling reduction enhancers, such as activated carbon, biochar, and zeolite, into AnMBR could enhance the reactor's removal performance and effectively alleviate membrane fouling as well (Chen et al., 2020; Chen et al., 2019a; Chong, 2015). Powdered activated carbon (PAC), which is comparatively simple and effective in its adsorption ability, has been broadly applied to aerobic and anaerobic MBRs for membrane fouling alleviation. The high adsorption capacity of PAC could result in significant removal of dissolved organic matter. Moreover, formation of biologically activated carbon (BAC) could facilitate aggregation of microorganisms as well as degradation of pollutants. The PAC addition to AnMBRs was able to remove COD, fine colloids, turbidity, colour, and antibiotics. It could also effectively mitigate membrane fouling by enlarging sludge floc size and decreasing SMP concentration (Baêta et al., 2016; Hu and Stuckey, 2007; Vyrides and Stuckey, 2009). Since the main obstacle of broader application of AnMBRs was reported as membrane fouling, it is necessary to study effective fouling control strategies in regard to energy consumption and costs. The addition of PAC can be energy effective due to the lack of rotation as well as cost effective Moreover, PAC has relatively cheaper price (0.15 AUD/g) than other chemical additives for fouling reduction. Thus, this study can be a good approach for assessment of economic feasibility of practical application of AnMBR with PAC addition.

Although several studies have investigated the PAC addition to AnMBRs, they mainly focused on removal performance of certain components such as colour, turbidity,

pharmaceuticals, or toxic compounds like aromatic amines. In addition, although some research reported membrane fouling reduction along with removal performances, they only paid attention to flux decline, concentration of SMP and EPS in mixed liquor, and sludge floc size. Few attentions was paid to the impact of PAC on microbial community in AnMBR, while microbial community might have close relation to membrane fouling behaviour. Since excessive dosage of PAC could lead to adverse results on membrane fouling due to the greater production of EPS and increased sludge viscosity, the optimisation of PAC dosage is necessary. Thus, a short-term study was firstly conducted to determine an optimal PAC dose by analysing the TOC, COD and nutrient removal, as well as membrane fouling regarding TMP development. A comparison study was then carried out to evaluate long-term effects on performance of AnMBR with the optimal PAC addition and AnMBR without PAC. Sludge characteristics, in terms of SMP and EPS, zeta potential, as well as membrane filtration resistance and microbial community were explored.

2. Materials and methods

2.1 Anaerobic sludge acclimatisation and characteristics of synthetic wastewater

The seed sludge used in this study was obtained from a local domestic sewage treatment plant in Sydney, Australia. The sludge during the start-up had mixed liquor suspended solids (MLSS) concentration of 7-8 g/L. Nitrogen was purged into the system using the diffused aeration tubes on a regular basis. The sludge was acclimatized for 90 days until the stable removal rates of total organic carbon and nutrient were gained.

The synthetic wastewater used in this study has feed COD concentration of 550 ± 10 mg/L and the C: N: P ratio of 100:5:1. Glucose ($C_6H_{12}O_6$), sodium nitrate ($NaNO_3$), and potassium phosphate (KH_2PO_4) were used as the major sources of carbon, nitrogen, and phosphorus

respectively, and 80% of $NaNO_3$ and 20% of NH_4Cl were mixed for nitrogen source. Magnesium sulphate ($MgSO_4 \cdot 7H_2O$), sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$), calcium chloride ($CaCl_2 \cdot 2H_2O$), manganese chloride ($MnCl_2 \cdot 7H_2O$), zinc sulphate ($ZnSO_4 \cdot 7H_2O$), ferric chloride anhydrous ($FeCl_3$), cupric sulphate ($CuSO_4 \cdot 5H_2O$), cobalt chloride ($CoCl_2 \cdot 6H_2O$), and yeast extract were used as trace nutrients.

2.2 Powdered activated carbon

PAC (Darco KB-B, Norit, US) was used in this study with the particle size ranging from 100 to 325 mesh and had 500–1000 m^2/g of surface area. PAC was first washed three times with milli-Q water and dried at 105 ^oC overnight before use. For determination of an optimal PAC dosage, a short-term study was implemented with three different PAC dosages, since the excessive dosing could cause more membrane fouling due to PAC being a potential foulant. While 2.5 of the PAC to biomass ratio with 5 g/L of PAC and 2 g/L of MLSS in the previous study showed the best flux improvement, the range of PAC dosages in recent studies regarding PAC addition to AnMBR was 0.4–5 g/L which had less than 1 of PAC to biomass ratio (Akram and Stuckey, 2008; Baêta et al., 2013; Chong, 2015; Park et al., 1999). Thus, the three PAC dosages for the optimisation were determined to be 1, 3, and 5 g/L, within the range of MLSS of the AnMBRs. PAC was dosed only at the initial stage of the experiment. Results on the optimisation of PAC are reported in section 3.1.

2.3 Experimental set-up and operating conditions

The two submerged AnMBR systems, which have working volume of 3.5 L were operated at the same time in the continuous mode. Hollow fibre membrane (Polyvinylidene fluoride

(PVDF), pore size 0.07–0.1 μm) with 1.00 mm of the inner diameter and 2.20 mm of outer diameter, and 0.08 m^2 of surface area, was used for this study. Before the PAC addition, the initial MLSS concentration were adjusted to 5 g/L, and mixed liquor volatile suspended solids/mixed liquor suspended solids (MLVSS/MLSS) ratio was over 0.8 in each reactor. Moreover, no excess sludge withdrawal was applied during each set of operation. The pH value and the temperature were kept constant at around 7.0 ± 0.1 and 22 ± 0.1 ℃, respectively. A constant pH value was maintained at around 7 by adding $NaHCO_3$ or H_2SO_4 to the feed solution. The two AnMBRs were operated at 5 L/m^2·h of flux. The synthetic wastewater was transported into each reactor using an influent pump at 6.67 ml/min of flow rate and an effluent pump was used as well at 8.33 mL/min of flow rate. The 8-minute suction and 2-minute relaxation mode was applied for the two AnMBRs. As the flow rate of influent was kept constant at 6.67 ml/min, the dilution rate in this study was kept at 0.114 h^{-1}. The determination of these experimental conditions was based on the previous study, which demonstrated that the highest VFA yield with simultaneous production of methane were achieved at 8 hrs of HRT, 550 g/L of COD and pH 7 (Khan, 2019).

The conventional AnMBR with the optimal PAC dosage was operated for a total of 67 days including chemical membrane cleaning two times. The membrane was chemically cleaned after the TMP reached over 35 kPa in each cycle. This value was based on the previous study which reported that the membrane required chemical cleaning when the TMP reached at 35 kPa (Deng et al., 2015). The first cycle maintained for 24 days, then 22 days in the second cycle, and the last cycle was operated for 21 days. On the other hand, AnMBR without PAC addition was operated for a total of 63 days including three chemical membrane cleanings, which maintained for 18 days in the first cycle, 18 days in the second cycle, 14 days in the third cycle and 13 days in the last cycle. Both reactors had no sludge withdrawal during the whole operation. Each operation cycle was followed by a one-week of recovery period after

the membrane cleaning to stabilize the pH level and temperature of the reactor so that a steady state could be remained in the next operation cycle. For the chemical cleaning of membrane, membrane was first placed in 0.5% of citric acid for 6 hours for removing inorganic foulant, and then put in 0.4% of sodium hydroxide for 6 hours to remove organic substances. Finally, it was put in 0.8% of sodium hypochlorite for another 6 hours for removal of microorganisms, bacteria, and algae. As soon as the membrane was separated from the reactor for the cleaning, nitrogen gas purging was applied for 5 minutes to remove unexpected oxygen and maintain the anaerobic condition in the reactor. While the membrane was immersed in the cleaning solution, the reactor was kept feeding with synthetic wastewater under anaerobic condition so that anaerobiosis could be maintained.

2.4 Analysis methods

2.4.1 Nutrient, COD, and sludge properties analysis

Measurements of MLSS and MLVSS concentrations were conducted by sieving a well-mixed sample with 1.2 μm filter paper. The remaining residue on the filter was dried in an oven at 105 ºC for 2 h. The increment in weight of the filter paper indicated the MLSS in the sample. Subsequently, the filter paper was ignited in a furnace at 550 ºC for 20 min. The weight reduced during ignition represented the MLVSS in the sample. Nutrient measurements, such as ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), and phosphate (PO_4-P), were carried out using the test kit (Merck Millipore, Burlington, USA) and a photometer (Spectroquant NOVA 60, Merck). The pH of the reactor was measured by using pH meter every day (HI9025, Hanna Instruments, Limena, Italy). Total organic carbon (TOC) concentrations of the influent and effluent were analysed using a TOC analyzer (Analytik Jena Multi N/C 2000). Chemical oxygen demand (COD) of influent and effluent was

analysed using COD test kit (HI93754B-25, Hanna Instruments Australia, Melbourne, Australia) and DR/2000 spectrophotometer (HACH) according to Standard Methods. For the measurement of zeta potential, 10 mL of the mixed liquor was extracted from each reactor at the end of the experiment, and Zetasizer (nano instrument ZS Zen3600, UK) was used for analysis.

2.4.2 Measurement of membrane fouling resistance

Membrane fouling resistance was measured to examine the filtration characteristics by using the resistance-in-series model, when J is the permeate flux, ΔP is the TMP, μ is the viscosity of the permeate (Deng et al., 2015):

$$J = \Delta P/\mu R_t$$

The total resistance, R_t, consists of three components, which are R_m, R_c, and R_p. It can be described as follows (Deng et al., 2014):

$$R_t = R_m + R_c + R_p$$

R_m is an inherent membrane resistance, R_c is a cake layer resistance which came from the deposited sludge particles on the membrane surface, and R_p is a pore blocking resistance which was brought about the adsorption of dissolved matters and colloids with similar size to membrane pore size (Deng et al., 2014). R_m was measured by using various fluxes with distilled water with a cleaned membrane before the operation. At the end of each operation, R_t was obtained with fouled membrane by the same methods as R_m. The cake layer was then removed using brush and distilled water from the membrane surface and $R_m + R_p$ was determined. R_c was calculated by deducting $R_m + R_p$ from R_t, and thus R_p could be calculated by deducting R_m from $R_m + R_p$.

2.4.3 Soluble microbial products (SMP) and extracellular polymeric substances (EPS)

The concentrations of proteins and polysaccharides in SMP and EPS from the mixed liquor were analysed based on the methods as described below. The 30 ml of mixed liquor was first centrifuged at 3000 rpm for 30 min after 30-min storage in the fridge. The supernatant was again centrifuged at 3000 rpm for 30 min, while the pellet was resuspended in 30 ml of phosphorous buffer solution. The second supernatant was sieved by 0.45 μm syringe filter, which was measured towards proteins and polysaccharides of SMP. The amount of cation exchange resin (Dowex^{TM} Marathon^{TM} C, Na^{+} form, Sigma-Aldrich, Bellefonte, PA) was calculated according to the MLVSS of the sample and then added on the resuspended pellet. This mixture was centrifuged at 2000 rpm for 2 hours, and the following supernatant was filtered by 1.2 μm syringe filter for the measurement of proteins and polysaccharides in EPS. Protein analysis were made according to modified Lowry method (Sigma, Australia), and polysaccharides were analysed based on Anthrone–sulfuric acid method (Deng et al., 2014).

The membrane module was taken out from the reactor after finishing the experiment for the analysis of SMP and EPS in the cake layer. The cake layer was collected by brushing the membrane surface, and then was dissolved in 30 mL of distilled water. Subsequently, the SMP and EPS of the collected cake layer were analysed by the same method as above.

2.4.4 DNA extraction and quality monitoring

The samples were taken out from each reactor and duplicated after the entire operation time for microbial community profiling. Samples were mixed with 100% ethanol (1:1 v/v) and stored at -20 ^{o}C before DNA extraction. Genomic DNA extraction was conducted using

QIAamp DNA Stool Mini Kit (Qiagen) by following the manuals. The concentration of the extracted DNA, its integrity and purity were analysed by NanoDrop® spectrophotometer. DNA concentrations of all samples were normalized to 20 ng/µl using DNase/Pyrogen-Free Water before the samples were sent to the sequencing facility.

2.4.5 Amplicon sequencing and bioinformatics analysis

The universal primer set Pro341F (5'-CCTAYGGGRBGCASCAG-3') and Pro806R (5'-GGACTACNNGGGTATCTAAT-3') was used to target both bacterial and archaeal 16S rRNA V3–V4 regions for depiction of the whole microbial community (Takahashi et al., 2014). Paired-end amplicon sequencing (2 × 300 bp) was conducted on the Illumina MiSeq platform (UTS Next Generation Sequencing Facility, Sydney, Australia). Raw sequence data were produced with the Illumina *bcl2fastq* pipeline.

Raw reads were brought into Quantitative Insights into Microbial Ecology (QIIME) 2 (version 2020.11.1) for computational analysis. Quality filtering, denoising (primer and read trimming), paired-end reads merging, dereplication, chimera filtering and amplicon sequence variants (ASVs) clustering (≥ 97% similarity) were performed using the *q2-dada2 denoise-paired* plugin. Reverse reads sequences were truncated at position 240 in the 3' end due to decrease in quality. Reads were mapped back to ASVs with a minimum identity of 97% to obtain the number of reads in each ASV.

Taxonomy was assigned to ASVs using the *q2-feature-classifier classify-sklearn* Naïve Bayes taxonomy classifier against the SILVA database (release 132) with a confidence of 0.7. All ASVs were aligned with mafft and used to construct phylogenetics tree with FastTree2 via the *q2-phylogeny align-to-tree-mafft-fasttree* pipeline. Alpha-diversity metrics

including Observed ASVs and Shannon index, beta diversity metrics including weighted UniFrac, unweighted UniFrac, Jaccard distance, and Bray-Curtis dissimilarity were estimated using *q2-diversity core-metrics-phylogenetic* pipeline after samples were rarefied (subsampled without replacement) to 18,000 sequences per sample.

3. Results and discussion

3.1 Determination of the optimal PAC dose

Three sets of experiments were conducted with different PAC dosage, which are 1, 3, and 5 g/L. As mentioned in section 2.2, the PAC dosages applied in the previous studies ranged between 0.4–5 g/L, with the PAC to biomass ratio of less than 1 (Hu and Stuckey, 2007). Since the excessive dosing could cause more membrane fouling due to PAC being a potential foulant, the highest PAC dosage was set to be 5 g/L, which was the MLSS concentration in this study. Thus, the three PAC dosages for the optimisation were determined to be 1, 3, and 5 g/L, within the range of MLSS of the AnMBRs. Each set of experiment was operated for a total of 25 days, including one chemical membrane cleaning during each operation, under the same operating conditions and set-up as the long-term study. As a result, AnMBR with 5 g/L of PAC revealed higher nitrate removal (78.3 ± 5.9%) and phosphate removal (21.8 ± 7.2%) compared to those with 1 g/L and 3 g/L of PAC addition. Overall, 5 g/L of PAC addition resulted in the improvement of nitrate removal rate and the lowest phosphate removal rate, which well described the anaerobic process. TOC removal performance showed slightly lower removal rate than the one with 3 g/L PAC addition, the average COD removal rate was the best in the 5 g/L experiment (61.1 ± 3.5%), compared to that of the 3 g/L experiment showed an average 55.2 ± 7.5%. In terms of TMP development, while 1 g/L and 3 g/L of PAC addition reached above 35 kPa on the 25th day of operation showing more rapid TMP

increase, 5 g/L of PAC addition presented almost 25 kPa. This could mean that membrane fouling has not been reduced due to the insufficient PAC doses, however, the highest PAC dosage facilitated more stable sludge properties by effective adsorption and subsequently enlarged floc size (Chong, 2015). In conclusion, based on the best nutrient and COD removals as well as membrane fouling control, 5 g/L of PAC was found to be the optimal dosage for conventional AnMBR, and consequently added for long-term operation of AnMBR.

3.2 Effect of PAC on the performance of AnMBR

According to the result from section 3.1 above, the conventional AnMBR with 5 g/L of PAC was operated for 67 days and the other AnMBR without PAC addition was operated in parallel for 63 days. Compared to the reactor with no PAC addition, 5 g/L of PAC showed approximately 7% higher nitrate removal rate (79.5 ± 4.6%) on average, and more stable and lower average phosphate removal efficiency (15.3 ± 5.4%) caused by abundance of denitrifying bacteria. The ammonium and nitrite removals in the reactor with PAC were 6.9 ± 5.7% and 3.3 ± 6.2%, respectively, which were almost 4% and 6% lower efficiencies than AnMBR without PAC. Overall, the optimal PAC addition resulted in more efficient nutrient removal rates including higher nitrate removal rate and the lowest phosphate removal rate, indicating the effective denitrification process.

AnMBR with one-off PAC dosing resulted in remarkably higher TOC removal rate than in the reactor with no PAC dosing, with the average rate of 75.5 ± 5.2% and 59.9 ± 6.9%, respectively. It was noticeable that the TOC removal rate significantly increased to almost 82.5% with the PAC addition during the initial 5 days, which might be resulted from the high adsorption capacity of PAC for organic matter. In terms of COD removal rate, the addition of

PAC resulted in around 15.7% higher COD removal rate (70.1 ± 2.0%) than no PAC addition (54.4 ± 4.7%) (Fig. 1a). Although the mixed liquor volatile suspended solids (MLVSS) concentration kept constant at 4.26 g/L before the operation, the increase of MLSS was observed in both AnMBRs. As soon as the PAC addition, the MLVSS concentration increased up to 9.24 g/L and at the end of operation, it was 11.43 g/L. The biomass concentration in AnMBR without PAC increased to 5.26 g/L after the entire operation. The PAC facilitated more biomass growth as it offered enough space to microorganisms to attach and thrive, thereby the biomass settleability was able to be improved.

TMP development is closely related to membrane fouling. Fig. 1b. presents the TMP development of the two reactors. Both AnMBRs started with 2.15 kPa and 2.55 kPa of TMP. However, there was a significant difference in time to reach up to 35 kPa. The AnMBR with PAC was operated for a total of 67 days including chemical membrane cleanings two times, while AnMBR without PAC was operated for 63 days including chemical membrane cleanings three times. The durations of first cycle were 18 and 24 days for the reactors with and without PAC respectively, and PAC addition provided 33% longer operation without chemical membrane cleaning. In the absence of PAC, the TMP increased rapidly with a sharp TMP jump within 5 days in each operational cycle. On the other hand, the presence of PAC showed a slow rise and TMP jump took place after 15 days of the operational cycle. The rapid TMP increase might be due to the accumulation of fine particles on membrane and pore clogging by biomass at the beginning of the operation (Chen et al., 2013; Pradhan et al., 2011). Moreover, after the chemical membrane cleaning, the TMP in AnMBR without PAC dropped to 3.11 kPa, whereas the TMP in AnMBR with PAC dropped to nearly 2.00 kPa, indicating an efficient restoration of membrane permeability. In terms of TMP increase trend for the last cycle in each reactor, it was shown that the rapid TMP increase occurred after 12 days with PAC addition while the increase occurred within 3 days without PAC addition. In

addition, as the last cycle was 3rd and 4th cycle for AnMBR with and without PAC respectively, the operation time lasted for 21 days in AnMBR with PAC, which was 8 days longer than that of AnMBR without PAC. Moreover, the operation time for the last cycle of AnMBR with PAC was shorter than the previous cycles because some amount of exhausted PAC might have acted as a foulant due to the one-off PAC dose at the first operation day. Thus, further investigation regarding the replenishment ratio as well as the different partial replacement ratios of PAC in long term operation can be carried out for the future study.

Fig. 1

3.3 Zeta potential in mixed liquor

It was investigated that hydrophobicity and surface charge could significantly influence the flocculation ability of sludge (Chen et al., 2018). Previous studies found that increased zeta potential and hydrophobicity enhanced flocculation of the mixed liquor and enlarged suspended flocs, thereby mitigating membrane fouling (Huang and Wu, 2008; Wu and Huang, 2010). In this study, the zeta potential value of anaerobic sludge in AnMBR with PAC was -11.60 ± 0.46 mV, while that in AnMBR without PAC was -14.40 ± 0.44 mV. The result showed that the PAC addition to AnMBR had higher zeta potential value than the conventional AnMBR, indicating that the negative charged sludge flocs could be reduced or that charge neutralization was caused by PAC. It has been reported that the charge neutralization by addition of flocculants could enhance the sludge flocculation ability (Deng et al., 2015; Ji et al., 2014). Hence, increase in zeta potential by PAC addition could attribute to improvement of biomass flocculation ability.

3.4 SMP and EPS in mixed liquor

The main fractions of SMP and EPS are mainly considered as proteins and polysaccharides. During the first 7 days of operation, the conventional AnMBR had higher total SMP and EPS values than AnMBR with PAC addition at 20.02 ± 0.83 mg/L and 22.94 ± 0.62 mg/L, respectively. While the AnMBR without PAC showed similar values of proteins and polysaccharides in SMP, concentrations of polysaccharides in SMP were much higher than those of proteins in AnMBR with PAC. The total EPS concentrations were twice as high in AnMBR without PAC as the one with PAC addition. After that, the SMP and EPS concentrations in AnMBR with PAC decreased during the 14 days of the operation. This was ascribed to that the addition of PAC, which has a high surface area, could initially reduce concentrations of both SMP and EPS by effective adsorption.

However, after the decrease in SMP and EPS concentrations in the second week of operation, the EPS concentrations in AnMBR with PAC remarkably increased up to 21.86 ± 2.00 mg/L. This might be attributed to the fact that PAC become saturated with organic matter and the subsequent attachment and abundant growth of microorganisms could release more EPS as microbial products. It was reported that EPS could enhance the flocculation ability of the mixed liquor by polymer entanglement, contributing to enlarged sludge floc size (Chen et al., 2018). Thus, this increase in EPS concentrations in AnMBR with PAC could enhance formation of larger flocs and sludge flocculation ability. Although it was reported that large amounts of EPS could accelerate the cake layer formation and fouling process, while excellent sludge flocculation and enlarged flocs might have rather formed a porous cake layer in this experiment (Chen et al., 2018). During the rest of the operational time, EPS concentration was slightly reduced to 18.32 ± 0.87 mg/L via adsorption and biodegradation by attached microorganisms on biologically activated carbon (BAC). Overall, the total EPS

concentrations in AnMBR with PAC were kept at lower values than the reactor without PAC. Infinite SRT in submerged AnMBR could result in high SMP concentrations (Huang et al., 2011). Although AnMBR with PAC was operated with infinite SRT, the SMP concentrations showed steady reduction over the whole operation period, indicating that PAC addition could effectively reduce SMP through the simultaneous adsorption and biodegradation.

On the other hand, in the AnMBR without PAC, total concentrations of SMP showed minor difference during the entire time, while there was a significant change in its composition. The amounts of polysaccharides in SMP significantly increased from 3.00 ± 0.26 mg/L to 17.01 ± 0.06 mg/L, while the amounts of proteins decreased. This could lead to severe pore blocking on the membrane due to the soluble state of SMP as well as the hydrophilic nature of polysaccharides (Chen et al., 2017). The total EPS concentrations incredibly increased, while the composition showed minor change during the whole time. The larger amounts of EPS could cause higher sludge viscosity and acceleration of cake formation, deteriorating membrane permeability (Chen et al., 2017).

Previous research found that the proteins to polysaccharides ratio of SMP and EPS (PN/PS ratio) played a significant role in membrane fouling, since the ratio might be closely related to properties of sludge flocs such as hydrophobicity and surface charge (Arabi and Nakhla, 2008; Tian et al., 2011). The higher PN/PS ratio of EPS was found in AnMBR with PAC compared to AnMBR without PAC after 21 days of operation. Higher level of proteins might have induced higher hydrophobicity and higher zeta potential of sludge, which enabled more agglomeration of sludge flocs. The PN/PS ratio of SMP remarkably increased up to 2.80, while that in AnMBR without PAC lowered after 14 days and stayed at lower values (0.06–0.36). This high fraction of proteins in SMP in AnMBR with PAC also induced better settleability by the hydrophobic nature of proteins. Previous research showed that high PN/PS

ratio of SMP had beneficial impact on fouling control, such as less irreversible fouling, and the results in this study also corresponded to this previous result (Yao et al., 2011). The time variations of SMP and EPS concentrations as well as composition in both AnMBRs are presented in Fig. 2.

Fig. 2

3.5 Membrane fouling behavior

The membrane fouling resistance of both AnMBRs in each cycle are presented in Table 1. Membrane fouling resistances were measured before and after each cycle when the chemical membrane cleaning was performed. Although the initial resistance of cleaned membrane (R_m) was similar in both reactors, the total resistance (R_t) was significantly higher in the AnMBR without PAC, indicating that the PAC addition could effectively reduce R_t. Moreover, R_t of both reactors showed gradually increasing trends, while that of AnMBR without PAC increased with a larger magnitude than the one with PAC addition. Since the ratios of cake layer resistance (R_c) to R_t in both reactors were between 76.17% and 86.44%, the predominant fouling mechanism in both AnMBRs might be due to cake layer formation. Pore blocking resistance (R_p) of AnMBR with PAC showed minor difference after all three cycles. However, R_p of AnMBR without PAC had a remarkable increase after four cycles, accounting for 10.78% of R_t after the entire operation. As discussed in 3.4, comparatively high SMP concentration in AnMBR without PAC could result in severe pore blocking due to the soluble form of SMP fractions (Chen et al., 2017). In addition, it was also noticeable that R_m of AnMBR without PAC increased after every membrane cleaning, while AnMBR with PAC remained at similar value, indicating that irreversible fouling has occurred without PAC addition.

Larger amounts of polysaccharides in SMP in mixed liquor might have induced more pore clogging due to hydrophilic property of polysaccharides, which could penetrate into the cake layer and then cause irreversible pore blocking (Deng et al., 2014). It was found that small sludge particles could lead to serious pore clogging and cake layer formation due to its high tendency to adhere on membrane surface and pore walls (Cheng et al., 2020). On the other hand, higher flocculation ability and PN/PS ratio of SMP induced by PAC addition brought about larger sludge flocs and subsequently less deposition on membrane surface, because of proportionally increased shear-induced diffusion and inertial lift force according to particle size (Pan et al., 2010). Therefore, PAC addition could prevent pore clogging and irreversible fouling on the membrane surface. Variations of membrane resistances in two AnMBRs are presented in Fig. 3.

Fig. 3

As the cake layer was the largest fraction of the total fouling resistance, the cake layer compositions of SMP and EPS were analysed. The EPS composition in the cake layer was similar in AnMBR with PAC and AnMBR without PAC in every cycle. On the other hand, the total SMP concentrations were remarkably higher with the absence of PAC (77.19 ± 7.37 mg/L) compared to the presence of PAC (17.43 ± 1.59 mg/L). In the SMP composition of the cake layer in AnMBR without PAC, the main fractions were proteins at the first cycle, accounting 51.78 ± 7.28 mg/L. From the second cycle, polysaccharides had larger composition than proteins, which were the main factor of cake layer formation as well as pore blocking, both leading to deterioration of membrane filterability. This result elucidated that R_c of AnMBR without PAC was mainly due to high SMP concentrations on the membrane surface. In AnMBR without PAC, large amounts of SMP could be adsorbed or attached on membrane surface because of the drag force from the effluent pump along with a rapid TMP increase (Deng et al., 2014). However, the PAC addition effectively reduced both proteins

and polysaccharides of SMP in cake layer, which might be resulted from the adsorption of SMP on PAC and biodegradation by attached microorganisms prior to attachment on membrane surface.

3.6 Impact of PAC addition on microbial community

3.6.1 Overview of sequencing data

Paired-end Illumina sequencing generated 203,054 sequences from 4 samples. At least 49.4% of input sequences of each sample passed quality filtering, 82.6% of filtered sequences were merged, and 99% of merged sequences were non-chimeric. The minimum number of sequences per denoised samples was 18,643, and the maximum number was 34,899. In total, 106,153 denoised sequences were clustered to a total of 751 ASVs with a mean frequency of 141.3. Rarefaction curves of Observed ASVs at maximum sequencing depth of 18,643 showed that all samples approached a saturation plateau at sequencing depth of about 17,000, confirming the sufficient sequencing depth in this study (Fig. 4).

Fig. 4

3.6.2 Impact of PAC addition on microbial diversity

AnMBR with PAC addition has a slightly lower diversity level compared to the reactor without PAC addition (Table 2). This is true for both species richness (number of observed ASVs) and species evenness (Shannon index). Low species richness and evenness indicated that PAC addition created a selective force, and some species were enriched that they became predominant microbes. To the best of our knowledge, the impact of PAC on microbial diversity in AnMBR has not been reported previously. Previous literatures showed that PAC

addition increased microbial diversity in conventional and dynamic aerobic MBRs (Asif et al., 2020; Hu et al., 2017). It is noteworthy that the microbial community in aerobic MBRs are very different from that in an anaerobic environment. Thus, further research is needed to elucidate more clearly how PAC affect microbial diversity in AnMBRs.

3.6.3 Impact of PAC addition on microbial composition

Microbial composition analysis revealed key functional groups in the anaerobic digestion process of both AnMBRs (Fig. 5). *Lactococcus* and unassigned *Enterobacteriaceae* were the two most abundant bacterial genera in all samples (24.8–30.6%). *Enterobacteriaceae* participates in hydrolysis and acidognesis while *Lactococcus* is a lactate producer (Detman et al., 2018). Other dominant hydrolytic and fermentative bacteria included unassigned *Saccharimonadaceae* (AnMBR without PAC), *Brooklawnia* (family *Propionibacteriaceae*), *Paludibacter*, *Thermovirga* (amino acid degradation), *Enterobacter*, *Lactobacillus*, and uncultured *Anaerolineaceae*. *Smithella* and uncultured *Synergistaceae* convert lactate and other volatile fatty acids to acetate – the precursor for methanogenesis performed by *Methanosaeta* (Liu et al., 1999, Wang et al., 2013).

PAC addition exerted strong impact on the microbial composition in AnMBR (Fig. 5 and Fig. 6), especially on filamentous and fouling-related microorganisms, which could reduce fouling propensity. Filamentous bacteria play an important role in wastewater treatment, as they can benefit the formation of flocs and subsequent settling by offering a base structure for other microorganisms to attach. However, excessive growth of filamentous bacteria can have negative effect on membrane fouling. Sludge viscosity increases due to the abundance of filamentous bacteria, which leads to the formation of non-porous cake layer on the membrane. More production of SMP by excess filamentous bacteria can also accelerate

severe membrane fouling (Deng et al., 2014). Filamentous microorganisms including uncultured *Anaerolineaceae*, *Chryseobacterium* and *Methanosaeta* can contribute to the initial attachment step of biofouling development due to their high affinity for attachment (Harb et al., 2015; Yamada et al., 2006). In fact, *Chryseobacterium* has been identified as one of three pioneer bacteria involving membrane biofilm development (Piasecka et al., 2012). Specifically, PAC addition could lead to lower relative abundance of filamentous microorganism in the reactor. The PAC addition might inhibit proliferation of filamentous bacteria and its high adsorption capacity could mitigate fouling by reducing the attachment of filamentous bacteria on the membrane surface. The relative abundance of these filamentous microbes in AnMBR without PAC was 1.7–4.7 times higher than that of AnMBR with PAC addition. PAC addition also resulted in lower relative abundance of fouling-associated bacteria such as *Cloacibacterium* and *Paludibacter* (Lei et al., 2019; Sun et al., 2016; Xu et al., 2020). *Cloacibacterium* was repeatedly detected in membrane biofilm, suggesting its high potential attachment on the membrane and membrane fouling propensity (Sun et al., 2016). Meanwhile, Lei et al. (2019) reported *Paludibacter* as one of the predominant bacteria in the fouling layer of AnMBR. On the other hand, the relative abundance of these fouling-associated bacteria was only 3.7–59.4% in AnMBR with PAC.

Fig. 5 and Fig. 6

In AnMBR with PAC addition, different non-filamentous microorganisms were enriched to fulfil functional roles of the above mentioned filamentous and fouling-related genera (Fig. 6). The role of uncultured *Anaerolineaceae* in hydrolysis and fermentation was met by *Microbacter* (relative abundance 7.1 times higher than AnMBR without PAC) (Harb et al., 2015), while *Treponema* (relative abundance 3.4 times higher) instead of *Smithella* was responsible for *acetogenesis* (Wang et al., 2013). PAC addition also resulted in a higher relative abundance of hydrogen/formate-utilizing methanogens *Methanoregula* and

Methanobacterium, as well as hydrogen-producing bacteria including *Enterobacter*, uncultured *Spirochaetaceae* and *Citrobacter* (Bräuer et al., 2011; Cabrol et al., 2017; Chen et al., 2019b; Thompson et al., 2008; Xiao et al., 2013). This indicated a higher contribution of hydrogenotrophic methanogenesis pathway to compensate for the lower abundance of acetoclastic methanogen *Methanosaeta*.

AnMBR with PAC showed an enhanced balance between archaeal and bacterial groups, with higher ratio between total relative abundance of methanogenic archaeal orders and bacterial genera (A/B) (0.083 compared to 0.075 in AnMBR without PAC). Higher balance among functional groups has been shown to be essential for stable and efficient process performance in AnMBR (Cheng et al., 2021). These results implied better COD and TOC removals observed with PAC addition. Genus *Zoogloea*, consisting of denitrifying bacteria was also enriched in AnMBR with PAC (Fig. 6), contributing to the higher nitrate removal efficiency observed (Huang et al., 2015). This was in agreement with findings from Asif et al. (2020) who reported an improved abundance of *Zoogloea* along with lower nitrate concentration in PAC-MBR compared to MBR without PAC.

4. Conclusions

The optimization of PAC dosage to a conventional AnMBR, as well as its long-term effect on removal performance and membrane fouling behaviour were investigated. The addition of the optimal PAC dosage to AnMBR effectively enhanced COD and TOC removal and decreased polysaccharide by high adsorption and biodegradation capacity. While the main cause of membrane fouling in AnMBR without PAC was pore blocking and irreversible fouling induced by SMP, the PAC addition could significantly prevent pore clogging and irreversible fouling by better sludge flocculation ability and higher hydrophobicity. These results were

also induced by declined abundance of filamentous and fouling-associated microorganisms.

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Table 1. Comparison of membrane fouling resistance values in two AnMBRs. (p.18, line 426)

	Membrane resistance[a] (m^{-1})	With 5 g/L PAC		Without PAC	
			% of R_t		% of R_t
1st cycle	R_t	9.25×10^{12}		1.52×10^{13}	
	R_m	1.60×10^{12}	17.29	1.66×10^{12}	10.92
	R_p	3.22×10^{11}	3.48	4.02×10^{11}	2.64
	R_c	7.34×10^{12}	79.35	1.34×10^{13}	86.44
2nd cycle	R_t	1.05×10^{13}		1.65×10^{13}	
	R_m	1.77×10^{12}	16.86	1.80×10^{12}	10.90
	R_p	4.16×10^{11}	3.96	6.51×10^{11}	3.95
	R_c	7.83×10^{12}	74.57	1.41×10^{13}	85.45
3rd cycle	R_t	1.15×10^{13}		1.72×10^{13}	
	R_m	1.79×10^{12}	15.56	1.91×10^{12}	11.10
	R_p	4.56×10^{11}	3.97	1.42×10^{12}	8.26
	R_c	9.24×10^{12}	80.35	1.39×10^{13}	80.81
4th cycle	R_t			1.93×10^{13}	
	R_m			2.20×10^{12}	11.40
	R_p			2.08×10^{12}	10.78
	R_c			1.47×10^{13}	76.17

[a] R_t, total resistance; R_m, cleaned membrane resistance; R_p, pore blocking resistance; R_c, cake resistance.

Table 2. Microbial diversity of two AnMBRs with and without PAC. (p.19, line 450)

Diversity index	Without PAC	With PAC
Number of observed ASVs	354.5 ± 79.5	296 ± 47
Shannon index	6.08 ± 0.23	5.92 ± 0.03

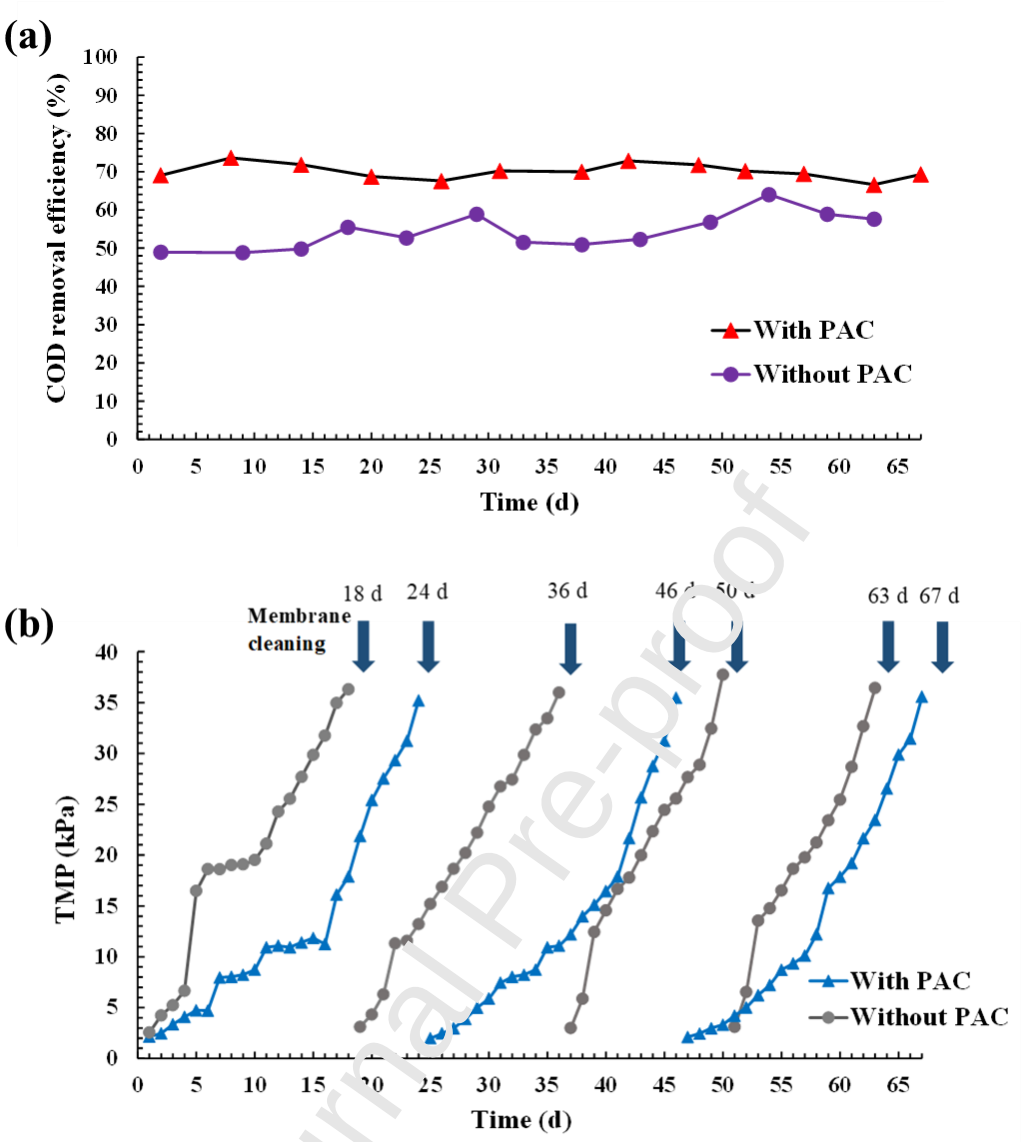

Fig. 1. The performance of two AnMBRs: (a) COD removal efficiency; (b) The variations of TMP during operation. (p.14, line 316)

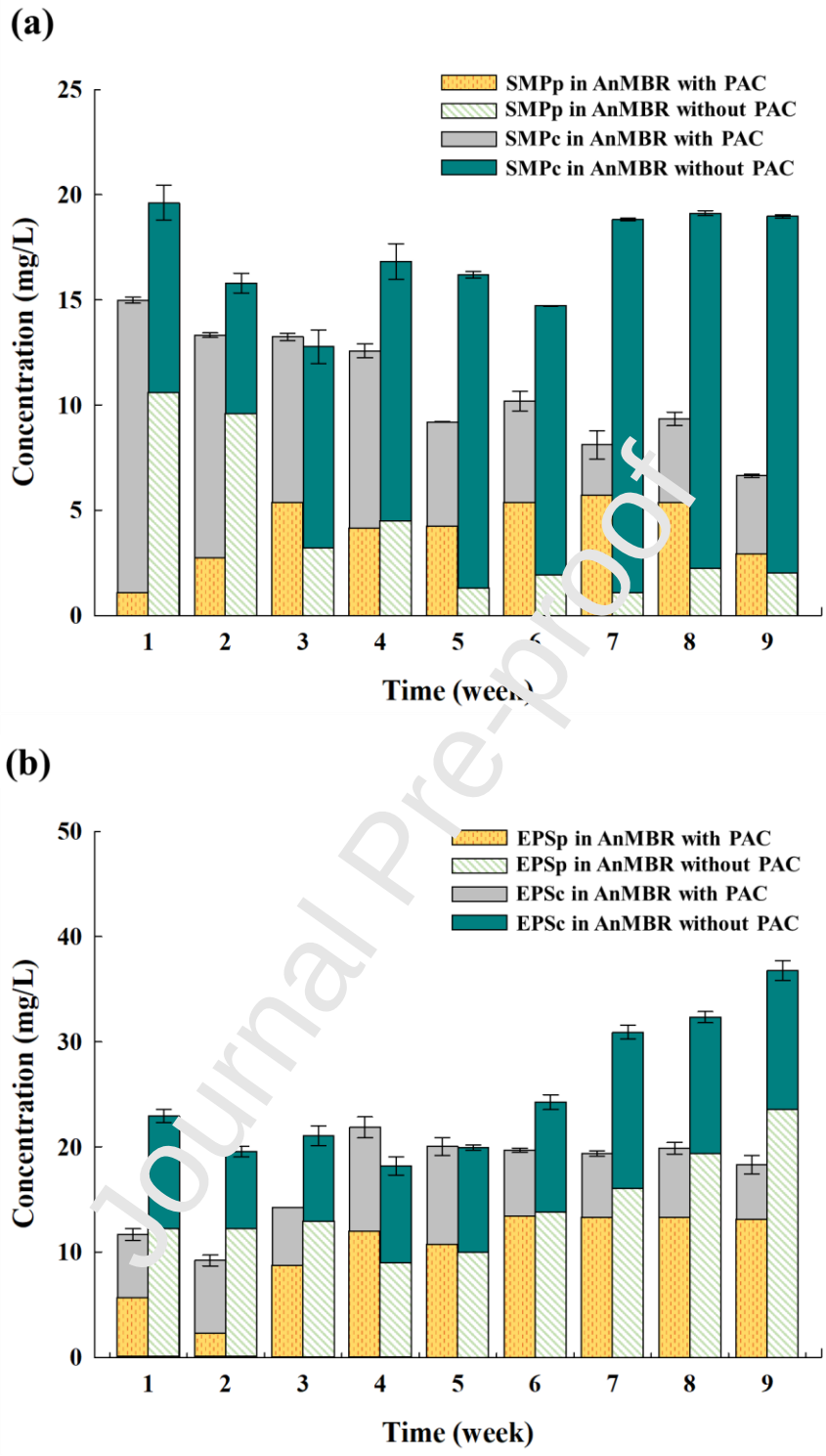

Fig. 2. (a) The variations of composition in SMP in two AnMBRs; and (b) The variations of composition in EPS in two AnMBRs (SMPp and SMPc indicate protein and polysaccharide in SMP, while EPSp and EPSc refer protein and polysaccharide in EPS, respectively). (p.16, line 382)

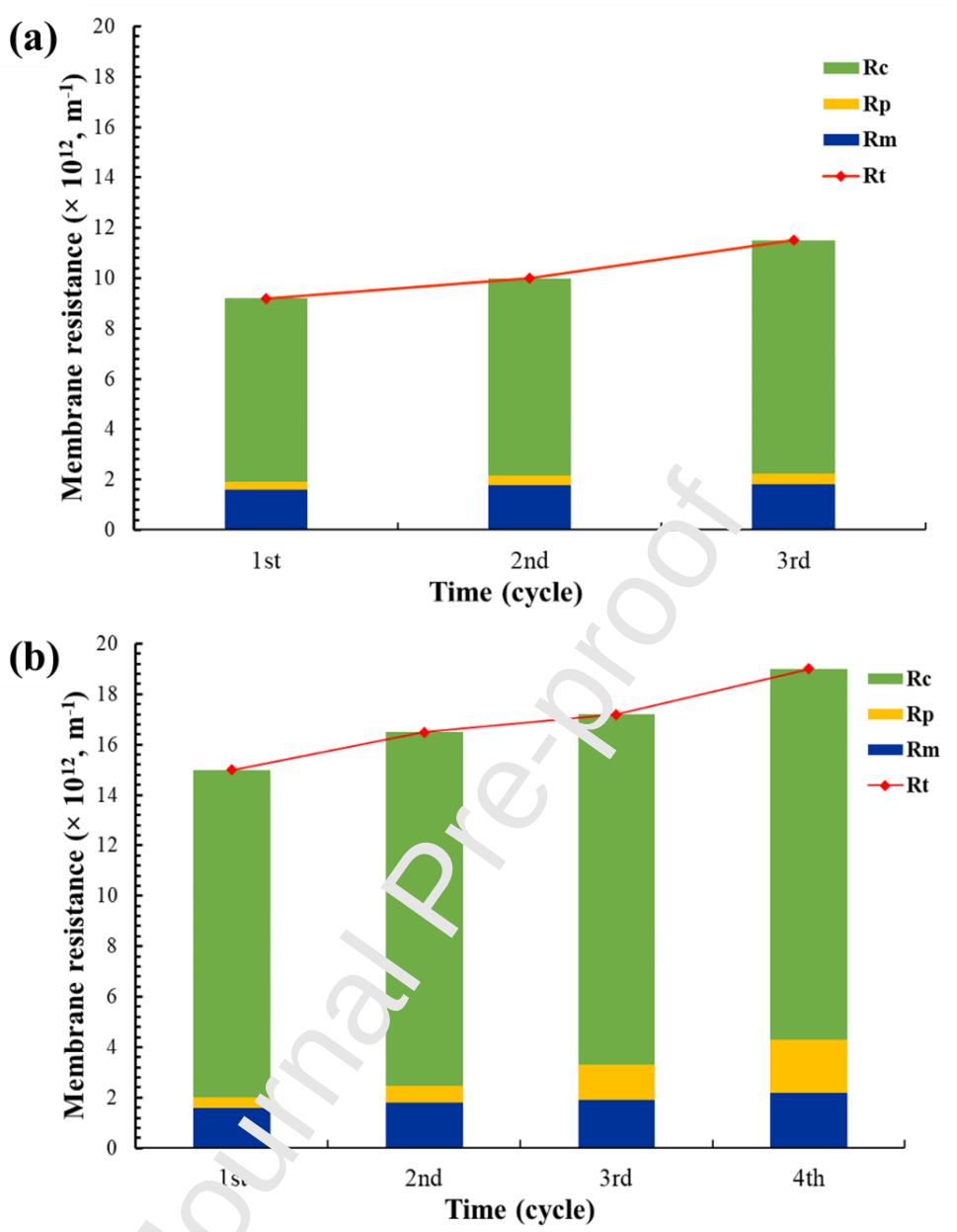

Fig. 3. The variations of membrane fouling resistance of two AnMBRs: (a) AnMBR with PAC; and (b) AnMBR without PAC. (p.18, line 427)

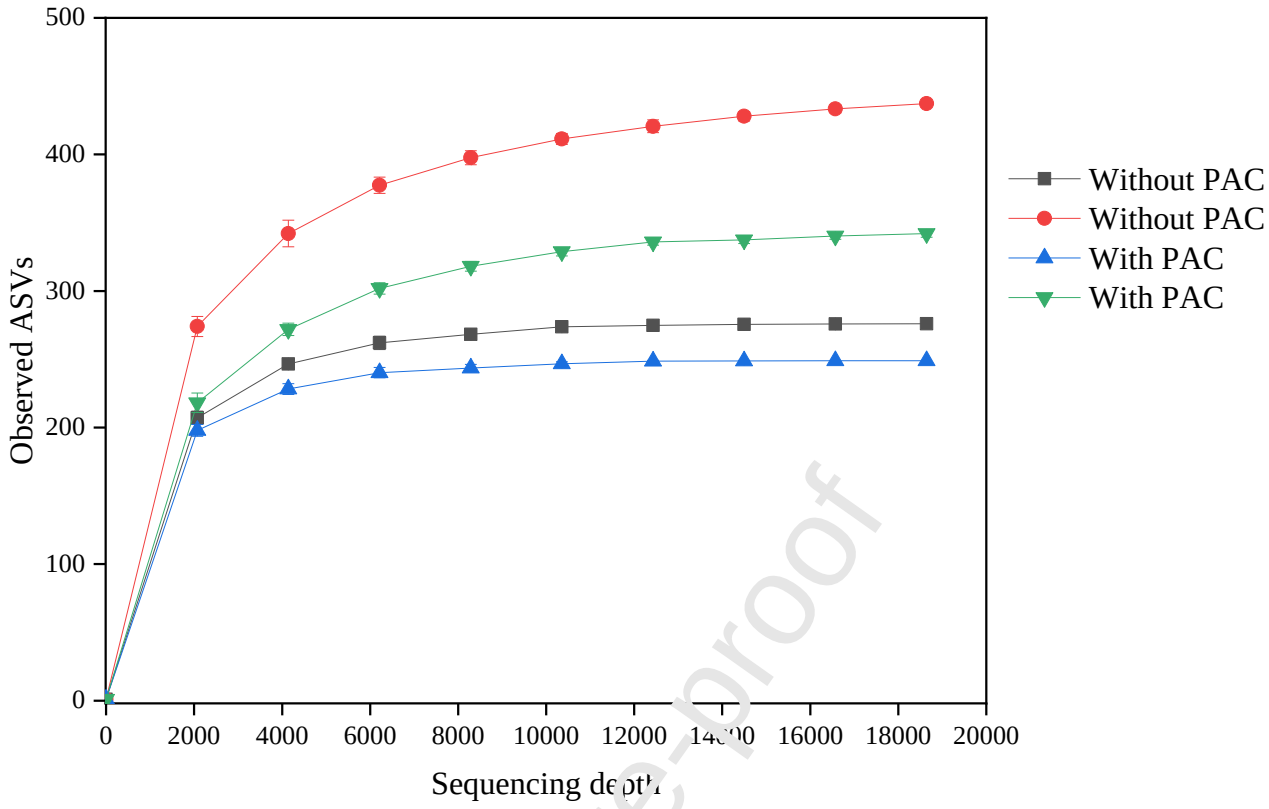

Fig 4. Rarefaction curves of 16S rRNA marker gene amplicon sequences at maximum depth of 18,043. (p.19, line 438)

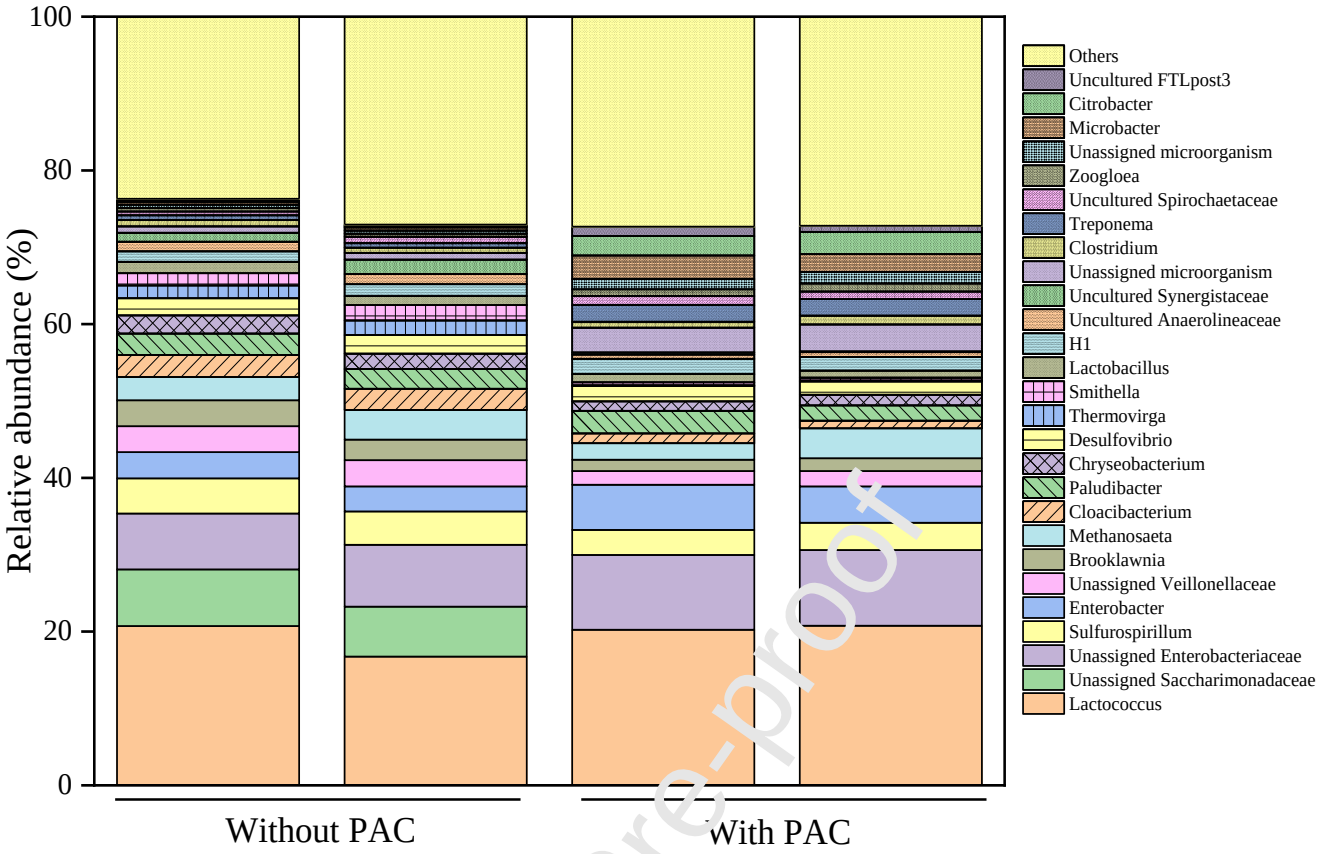

Fig. 5. Microbial composition in AnMBRs with and without PAC addition. Data are presented at genus level. Only genera with relative abundance > 1% in at least one sample are shown individually, the rest are grouped into "Others". (p.22, line 508)

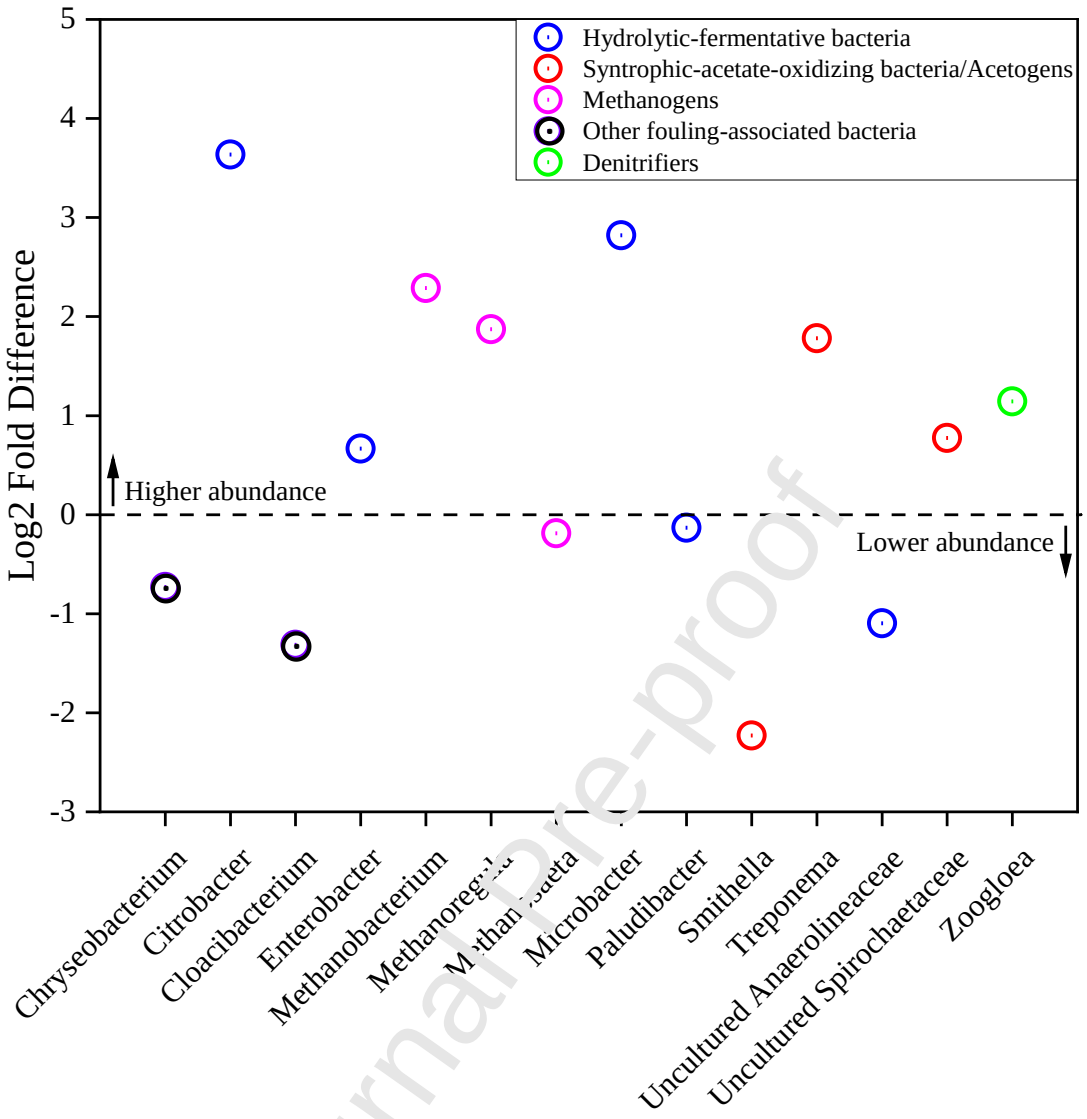

Fig. 6. Impact of PAC addition on microbial composition represented by log2-fold difference in the relative abundances at genera level, with genera coloured by functional groups. (p.22, line 509)