# Effect of magnetic powder on membrane fouling mitigation and microbial community/composition in membrane bioreactors (MBRs) for municipal wastewater treatment

Yi Liu[a,b,1], Qiang Liu[b,1], Jixiang Li[a,c]*, Huu Hao Ngo[d], Wenshan Guo[d], Jiajun Hu[e], Min-tian Gao[e], Qiyuan Wang[f], Yuansheng Hou[g]

[a]*Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China*

[b]*Shanghai University, Shanghai 200444, China*

[c]*University of Chinese Academy of Sciences, Beijing 100049, China*

[d]*Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NWS 2007, Australia*

[e]*Shanghai Key Laboratory of Bio-Energy Crops, School of Life Sciences, Shanghai University, Shanghai 200444, China*

[f]*FuXing Senior High School, Shanghai 200434, China*

[g]*QingHai Salt Lake Industry Company Limited, Geermu 816000, China*

[1]equal contribution.

*Corresponding author: Tel.: +86 21 20325167. E-mail: lijixiang@sari.ac.cn (J.X. Li) or 13524516063@163.com (J.X. Li).

**Abstract:** This study aims to investigate the usefulness of magnetic powder addition in membrane bioreactors (MBRs) for membrane fouling mitigation and its effect on microbial community and composition. The comparison between the two MBRs (one with magnetic powder (MAS-MBR) and one without magnetic powder (C-MBR)) was carried out to treat synthetic municipal wastewater. Results showed that bioflocculation and adsorption of magnetic powder contributed only minimally to membrane fouling mitigation while the slower fouling rate might be ascribed to magnetic bio-effect. The macromolecules (larger than 500 kDa and 300-500 kDa) of soluble microbial product from the MAS-MBR were reduced by 24.06% and 11.11%, respectively. High-throughput sequencing demonstrated the most abundant genera of biofilm sludge indicated lower abundance in bulk sludge from the MAS-MBR compared to the C-MBR. It is possible that less membrane fouling is connected to reductions in large molecules and pioneer bacteria from bulk sludge.

**Keywords:** membrane fouling, magnetic powder, microbial community, adsorption, bioflocculation

## 1. Introduction

Membrane fouling, especially biofouling, has severely hindered the widespread application of membrane bioreactor (MBR), and in fact it dramatically undermines the overall membrane performance. Occurring problems are severe flux decline, increased operating costs and frequent membrane replacement (Chen et al., 2015; Deng et al., 2016). To date the greatest challenge in MBR operation is overcoming the serious problem of membrane fouling.

Several methods have been used to alleviate membrane fouling in MBRs by modifying membrane materials, optimizing operational conditions and improving sludge properties of mixed liquors (Aslam et al., 2017; Ye et al., 2017; Zhou et al., 2014). Since the first two methods always increase membrane price or maintenance costs, adding coagulants, absorbents or carriers to MBRs to improve sludge properties of mixed liquors has been done extensively to mitigate membrane fouling (Chen et al., 2017; Miao et al., 2017). Baygi et al. (2016) reported that concurrent $KMnO_4$ oxidation and polyelectrolyte flocculation could increase the sludge particle size, reduce the soluble microbial product (SMP) concentration and alleviate the biofilm layer resistance. Xia et al. (2016) found that added bamboo charcoal (BC) prolonged operating time by decreasing SMP concentration and reducing membrane resistance. Deng et al. (2014) developed a sponge-submerged membrane bioreactor (SSMBR) that maintained trans-membrane pressure (TMP) at 2.0 kPa over a period of 90 days by: firstly, depressing the bound extracellular polymeric substances (EPS) concentration; and secondly, improving the particle size, zeta potential and relative hydrophobicity of sludge floc. However, it should be noted that while a bio-carrier can scour membrane biofilm, it may also lead to the breakup of activated sludge or damage to membrane. Meanwhile, it is known that organic or inorganic additives can increased bioreactor performance and reduced biofouling to some extent, however, they may cause "secondary pollutants" during wastewater reclamation and reuse (Deng et al., 2016). Moreover, bio-carriers lose activity over time and must be supplemented with fresh materials. It is evident that chemical waste sludge is difficult to handle, and in fact, the cost of anti-fouling by chemical additives is potentially high.

Unlike the methods above, the magnetic activated sludge (MAS) process has great potential for mitigating membrane fouling because the magnetic materials are inert and biocompatible, and furthermore are not detrimental to biological activity (Wang et al., 2016). The responsiveness of magnetic materials to the magnetic field enables their separation, reactivation and reuse. The generation of complex chemical waste sludge in one study was avoided (Zhou et al., 2015). With these properties, magnetic particles have already been added to biological reactors to improve their performance (Ma et al., 2017), whereas they have seldom been employed as fouling reducers in MBRs (Liu et al., 2017). The knowledge of the sludge properties, microbial community as well as their connection to membrane fouling of added magnetic particles in MBRs is still limited. Also, the anti-fouling mechanisms of magnetic particles fulfilled by adsorption, bioflocculation or magnetic bio-effect have not been well examined and still need to be verified.

Hence, in this study, a comparison was conducted to evaluate the performance of a magnetic MBR (MAS-MBR) and a conventional MBR (C-MBR) based on pollutants removal, sludge properties and membrane fouling. High-throughput sequencing technology served to compare the microbial communities of bulk sludge and biofilm sludge in both MBRs at the phylum, class and genus level, in order to link microbial community and membrane fouling. Possible anti-fouling mechanisms of magnetic particles were also examined and discussed. It is anticipated that this study will provide a deep and practical insights into the mechanism of membrane fouling mitigation and consequently advance our knowledge on the corresponding fouling control strategy by adding magnetic powder in MBRs.

## 2. Materials and methods

### *2.1. MBR descriptions and operating conditions*

Two lab-scale aerobic MBRs were used in this study with the same effective volume of 4 L. One was a MAS-MBR with 1g/L added magnetic powder, and the other was a C-MBR without magnetic powder added. The average particle size of magnetic powder ($Fe_3O_4$) used in this analysis was 9.119 μm (Sinopharm Chemical Regent Co., Ltd, China). Both MBRs were operated under the same hydraulic retention time (HRT) of 8 h. For each MBR, a hollow-fiber polyvinylidene fluoride (PVDF) membrane module with a total surface area of 0.044 $m^2$ was used. The nominal membrane pore size was 0.1 μm and the outside and inner diameters of the fibers were 1.0 and 0.85 mm, respectively. The reactors were seeded with aerobic activated sludge from a local municipal wastewater plant, and fed with synthetic wastewater (glucose, 175 mg/L; corn starch, 175 mg/L; $NH_4Cl$, 300 mg/L; $KH_2PO_4$, 52.8 mg/L; $Na_2CO_3$, 700 mg/L; $CaCl_2$, 8 mg/L; $MgSO_4 \cdot 7H_2O$, 9 mg/L; $FeSO_4$, 0.3 mg/L and trace elements). The synthetic wastewater was prepared every day. Feed wastewater and operational conditions of both MBRs are summarized in Table 1. Air was supplied continuously under the membrane module at a flow rate of 0.1 $m^3$/ h for the entire duration. Permeate was extracted from the membrane at a constant permeate flux of 11.25 L/$m^2$ h by a peristaltic pump with an intermittent suction cycle of 9 mins on and 3 mins off. Trans-membrane pressure (TMP) was recorded using a vacuum meter to monitor the evolution of membrane fouling. When the value of the vacuum meter reached 35 kPa, the membrane module was removed for cleaning. Chemical cleaning was done by soaking the membrane modules in solutions of 1% hydrochloric acid for 2 h and 0.2%

sodium hypochloride for 12 h. No excess sludge was discharged during both MBRs' operation, except for samples taken out to measure the suspended solids. The experiment temperature was conducted at 23.7 ± 1.7 $^{o}$C.

*2.2. Analytical methods*

The feed and permeate of both MBRs were sampled every day and analyzed for chemical oxygen demand (COD) and ammonia nitrogen ($NH_4^+$-N) by a HACH DR2800 spectrophotometer (USA) with original reagent kits. The mixed liquor suspended solids (MLSS) was measured according to Chinese NEPA standard methods (2002). The pH value and dissolved oxygen (DO) were measured by a portable digital multifunctional meter (Hach HQ40d, USA). The particle size distribution (PSD) of sludge samples in the reactors were measured on a static light scattering particle size analyzer (Malvern 2000, United Kingdom). The molecular weight (MW) was characterized by a gel permeation chromatography (GPC) system (Shimadzu LC-20AD, Japan). While sludge activity was measured using the triphenyltetrazolium chloride (TTC) method (Xia et al., 2010).

SMP and EPS were extracted based on a modified thermal method as suggested by Xia et al. (2010). Briefly, 40 mL of mixed liquid from each reactor was centrifuged for 8 mins at 6000 g and the supernatant was collected for SMP analysis. The retained pellet was resuspended with 0.9% NaCl solution. After necessary vortex oscillation, 8 mins ultrasonication and 10 mins vibration followed, and then the resuspended pellet was centrifuged for 10 mins at 8000 g. The supernatant was collected for loosely bound EPS (LB-EPS) analysis. Next, the retained pellet was resuspended again with 0.9% NaCl solution and this was followed by: vortex oscillation, 4 mins ultrasonication, 30 mins

thermal treatment at 80 °C and 20 mins centrifugation at 12000 g. Finally, the supernatant was collected for tightly bound EPS (TB-EPS) analysis. The concentrations of SMP and EPS were quantified as carbohydrates and proteins, respectively. Carbohydrates and proteins were quantified using the anthrone method with glucose as a standard and bicinchoninic acid assay (BCA) with bovine serum albumin as a standard, respectively.

*2.3. Magnetic powder adsorption batch test*

Prior to the adsorption test, the pure magnetic powder was washed three times with distilled water and dried for 4 h in an oven. For the adsorption test, 0.2 g dry magnetic powder was added to 20 mL of extracted SMP and LB-EPS, and then shaken at 150 g and 25 °C for 8 h. After that the samples were subjected to centrifugation at 5000 g for 8 mins to separate the magnetic powder. The raw solution and supernatant were taken for quantified analysis of proteins and carbohydrates as described in section 2.2.

*2.4. Microbial diversity analyses*

*2.4.1. DNA extraction and PCR amplification*

In order to comprehensively analyze the microbial communities, 3 activated sludge samples were collected from the seed sludge, C-MBR and MAS-MBR, respectively. Additionally, the biofilm samples were taken from the fouled membranes using a sterile steel knife. To more effectively release the bacterial cells sticking to the membranes, an additional bead-beating step (5 mins) was included before DNA extraction. Microbial DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, Georgia, U.S.) according to the manufacturer's protocols.

For high throughout sequencing analysis, PCR was executed with primers 515F

(5'-barcode-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRA GTTT-3') as follows: 95 $^{o}$C for 3 mins, followed by 27 cycles at 95 $^{o}$C for 30 s, 55 $^{o}$C for 30 s, and 72 $^{o}$C for 45 s and a final extension at 72 $^{o}$C for 10 mins. PCR reactions were conducted in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

*2.4.2. Illumina MiSeq sequencing and bacterial community analyses*

The amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, California, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, U.S.). Purified amplicons were pooled in equimolar and paired-end sequenced (2×250) on an Illumina MiSeq platform according to the standard protocols. Then raw Fastq files were de-multiplexed, and then quality-filtered using QIIME (version 1.9.1). Operational Units (OTUs) were clustered with 97% similarity using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP090271). Rarefaction curves, community richness estimators of Chao 1 and Ace, community diversity estimators of Shannon, Simpson and Good's coverage were generated with MOTHUR for each sample. Following phylogenetic allocation of the sequences down to the phylum, class and genus level, relative abundance of a given phylogenetic group was set as the number of sequences affiliated with that group divided by the total number of sequences per sample. Hierarchical cluster (Heatmap) analysis was performed using R-project gplots

(http://www.r-project.org/) in Linux.

## 3. Results and discussion

### *3.1. The performance of two MBRs*

Table 2 summarizes the COD and $NH_4^+$-N removals in the C-MBR and MAS-MBR during the operation. It can be seen that both MBRs achieved COD removal efficiency of more than 85% and $NH_4^+$-N removal efficiency greater than 90%, with the MAS-MBR indicating slightly better performance. It can therefore be concluded that adding magnetic powder in the MBR had no negative impact on COD and $NH_4^+$-N removals. Similar results were reported by Ying et al. (2010) and Ma et al. ( 2017). Herein the indistinct advantage of removing pollutants from the MAS-MBR may be due to the inherent better performance of MBR.

Although the MAS-MBR did not present superior pollutants removal in comparison with the C-MBR, it presented an obvious positive impact on membrane fouling mitigation. The TMP profiles of two MBRs confirmed it. During the operation, the TMP in the C-MBR reached above 35 kPa until day 28 while the TMP in the MAS-MBR reached above 35 kPa until day 48. These results indicated that adding magnetic powder could significantly mitigate membrane fouling, which may be attributed to the variation of SMP and EPS concentrations and compositions (referring to the details in Section 3.4). A similar result was reported by Wang et al. (2016).

### *3.2. Mixed liquor suspended solid concentration and particle size distribution*

Initially, the MLSS concentrations of both MBRs were 4.59 ± 0.29 g/L. During the operations, sludge concentrations kept increasing in both MBRs on account of no sludge waste. At the end of each operation, MLSS concentrations of the C-MBR and

MAS-MBR increased slightly to 4.99 ± 0.4 g/L and 4.92 ± 0.38 g/L, respectively. Obviously, MLSS concentrations in both MBRs were maintained at low levels, which may be attributed to the fewer organic compounds in the feed wastewater and intense aeration in the reactors (Xia et al., 2010).

To detect the degree of flocculation, the sludge size distributions were tested. Results of particle size distribution showed that sludge mixtures from the C-MBR (50.97±8.00 μm) and MAS-MBR (51.65±7.15 μm) had similar floc size during the operation. It is obvious that adding magnetic powder had little effect on sludge flocculation, consequently contributing to a minimal effect on fouling mitigation. Semblante et al. (2013) in their study documented similar results, and they explained the fouling mitigation as being caused by the adsorption of magnetite.

*3.3. Effect of magnetic powder adsorption on membrane foulants*

To confirm the adsorption effect of magnetic powder on membrane foulants, batch adsorption tests were performed with the real extracted SMP and EPS solutions in triplicate. Results showed that the average SMP concentrations before and after adsorption were 28.66 ± 1.22 mg/L and 26.16 ± 0.87 mg/L, respectively, while the average LB-EPS concentrations before and after adsorption were 51.58 ± 2.54 mg/L and 48.47±1.78 mg/L, respectively. The averaged calculated adsorption capacity for SMP and LB-EPS were only 0.25 mg/g and 0.31 mg/g after 8 h adsorption, respectively, which might be ascribed to the small BET specific surface area of 1.54-2.72 $m^2$/g for the added magnetic powders. Those results implied that the micro-sized magnetic powders used in this study had little effect on foulants' adsorption, and contributed to minimal impact on mitigating membrane fouling. Semblante et al. (2013) discovered

that porous magnetite with a specific surface area of 130-180 $m^2/g$ did not possess significant adsorption capacity when using real membrane foulants on account of there being little affinity. Thus further research is needed to verify the impact of magnetic powder on biomass properties, microbial communities and the parts they play in membrane fouling.

*3.4. Effect of magnetic powder on SMP and EPS*

According to previous reports, SMP and EPS have been widely considered as the main membrane foulants in MBRs (Deng et al., 2016; Meng et al., 2017). Herein polysaccharides and proteins as the major components of SMP and EPS were analyzed periodically from the mixed liquors of each MBR. Also, EPS was further divided into LB-EPS and TB-EPS. Figure 1 compares the compositions of SMP and EPS from the mixed liquors in the two MBRs. As expected, the average SMPc and LB-EPSc in the MAS-MBR were lower than that of the C-MBR while SMPp and LB-EPSp emerged as synchronisms. Nonetheless, the proteins and polysaccharides of TB-EPS appeared as very contrasting trends. Taking into account the occurrence of severe membrane fouling in the C-MBR, it can be concluded that SMP and LB-EPS played an important role in membrane fouling whereas TB-EPS was only weakly linked to biofouling. Similar results were reported in other studies (Meng et al., 2011; Seviour et al., 2012). As described in sections 3.2 and 3.3, bioflocculation and foulants adsorption of magnetic powders had a minimal effect on fouling mitigation. Thus it could be inferred that the lower-rate fouling in the MAS-MBR might be ascribed to magnetic bio-effect, which reduces SMP and LB-EPS production or alters the compositions of mixed liquors. It has been reported that the variations of EPS and SMP simply reflect changes in the

microbial community (Ding et al., 2016). Consequently, it is necessary to compare both MBRs' microbial communities.

On the other hand, the high microbial activity caused by magnetic induced effect, supported the conclusion. The average dehydrogenase activity of activated sludge in the MAS-MBR increased from 7.4 mg/L to 42.0 mg/L compared to that of the C-MBR. Wang et al. (2016) noted that adding 120 mg/L of magnetic powder could significantly improve dehydrogenase activity and encourage microbes to produce less SMPc and EPSp, resulting in curtailed membrane fouling. However, Ma et al. (2017) found that $Fe_3O_4$ NPs made activated sludge more toxic and destroyed the integrity of microbial cytomembrane in a sequencing batch reactor (SBR). These contradictory conclusions may be due to the different properties of magnetic particles. The added magnetic particles used in Wang et al.'s (2016) and our studies were micro-size magnetite whereas those used by Ma et al. (2017) were magnetic $Fe_3O_4$ nanoparticles. For this reason further research should be conducted to clarify the impact of different size magnetic particles on the microbes and microbial metabolites, plus their effects on membrane fouling.

*3.5. Effect of magnetic powder on molecular weight distribution and transformation*

It has been well recognized that large molecules played a more important role in membrane fouling compared to small molecules (Deng et al., 2016; Yang et al., 2010). Also, it is reported that microbial metabolites such as SMP and EPS could be effectively eliminated via biodegradation in MBRs (Hu et al., 2016; Meng et al., 2009). GPC therefor was used to compare the MW distributions of SMP, EPS and MBR permeate in both MBRs to further reveal the influence of magnetic bio-effect on microbial

metabolites and membrane fouling.

Figure 2 shows the MW distribution and transformation at different stages in both MBRs. It can be seen that only a small amount of organics with MW <100 kDa of SMP and MBR permeate presented in both MBRs, while the macromolecules with MW > 100 kDa were the major components, adding up to more than 98% of the total organics. Moreover, the proportions of the macromolecules with MW > 500 kDa and 300-500 kDa in SMP were larger than those in MBR permeate. This also suggested that macromolecules were more prone to membrane fouling. In contrast, the majority (~ 90%) of LB-EPS and TB-EPS in both MBRs were below 100 kDa, in which the organics with MW 10-100 kDa constituted the major component, ranging from 74.01% to 77.04% of the total organics. It should be noted that SMP featured generally larger macromolecules than EPS, and consequently played a more important role than EPS in membrane fouling (Aslam et al., 2017). It is interesting that adding magnetic powder in the MBR significantly decreased the percentages of macromolecules of SMP whereas had little impact on MW distributions of EPS and MBR permeate. As shown in Figure 2, the percentages of large molecules (MW >500 and 300-500 kDa) for SMP from the MAS-MBR were smaller (by 24.06% and 11.11%, respectively) than those of the C-MBR. It happened that latter and slower membrane fouling occurred in the MAS-MBR compared to the C-MBR. This might be ascribed to magnetic bio-effect, which could change the microbial community/composition and microbial activity in the MBRs. The large molecules could be successfully transformed into small ones via the superior biodegradation capacity of the microbes in the MAS-MBR, further resulting in less membrane fouling.

*3.6. Effect of magnetic powder on microbial community and composition*

*3.6.1. Microbial diversity and richness*

It is well known that the properties of mixed liquors depend on the microbial community, and variations in EPS and SMP simply reflect changes in the microbial community (Ding et al., 2016). Thus high-throughput sequencing served to characterize the microbial community and composition in the MBRs and provided deep insights into the effect of added magnetic powder on the microbial community and membrane fouling mitigating.

Five 16S rRNA gene libraries were constructed from bulk sludge and biofilm sludge of two MBRs. A single sample was collected from the inoculated sludge (C0), and four samples were collected from bulk sludge and biofilm sludge of two MBRs, including two bulk sludge samples from the C-MBR (C) and the MAS-MBR (M), and two biofilm sludge samples from the C-MBR (C-bf) and the MAS-MBR (M-bf) at the operation's end. After necessary denoising and filtering, the effective reads from each sample were used for further analyses. The diversity estimators of Chao 1, Ace Shannon, Simpson and Good's coverage for each sample are illustrated in Table 3. In this study, the Good's coverage of all samples was above 0.998, indicating that the sequencing depth could reflect the real microbial community for both MBRs. It was apparent that the microbial community and composition of the inoculated sludge were quite different from all the MBR samples, and the microbial diversity and richness of the inoculated sludge were far lower than those of MBR samples. This could be attributed to membrane rejection of microbes in MBRs. In both MBRs the microbial richness (estimated by Chao 1 and Ace) and diversity (estimated by Shannon and Simpson) of bulk sludge were lower than that

of biofilm layer. Thus it could be implied that the microbial community of biofilm sludge had greater richness and more diversity than the bulk sludge in both MBRs. It is also worth noting that the general microbial diversity and richness of bulk sludge in the C-MBR and MAS-MBR were similar, and the biofilm sludge emerged as synchronisms. Consequently, further comparison of the microbial composition was needed to reveal more information on the microbial community differences between the C-MBR and MAS-MBR.

*3.6.2. Comparison of microbial phylum, class and genus*

To better understand the effect of added magnetic powder on the microbial community and composition and how to mitigate membrane fouling, the sequence reads obtained from Illumina MiSeq sequencing were systematically analyzed at the phyla, class and genus levels.

At the phylum level (Figure 3 (a)), *Proteobacteria* and *Bacteroidetes* were the absolutely dominant phyla in the bulk sludge and biofilm sludge derived from both MBRs. The other dominant phyla present as ≥1% of the sequence reads also included *Planctomycetes*, *Acidobacteria*, *Firmicutes*, *Actinobacteria*, *Nitropirae*, *Chlorobi*, *Chloroflexi* and *Verrucomicrobia*. They have been reported to be ubiquitous in both lab-scale and pilot-scale membrane bioreactors (Chen et al., 2015; Ding et al., 2015; Fykse et al., 2016). With reference to bulk sludge, it is interesting that *Proteobacteria* increased from 30.58% to 50.60% while *Bacteroidetes* decreased from 35.60% to 24.30% in the MAS-MBR compared to the C-MBR. It has been reported that *Proteobacteria* includes a variety of functionally important bacteria for organic and nitrogen removal (Qi et al., 2016). Thus the higher abundance of *Proteobacteria* could account for the

slightly better removal of pollutants in the MAS-MBR than the C-MBR. Ding et al. (2016) asserted that the larger amount of *Proteobacteria* supported the slightly higher removal of nutrients in a forward osmosis MBR for anaerobic effluent treatment. More importantly, it may be associated with the transformation of SMP from large molecules to small molecules in the MAS-MBR, which resulted in the slighter membrane fouling.

*Bacteroidetes* have been widely reported as involved in membrane fouling (Ding et al., 2016; Guo et al., 2015; Xia et al., 2010). First, it is reported that *Bacteroidetes* could potentially release more membrane foulants such as proteinaceous EPS (Gao et al., 2010). Also, *Bacteroidetes* possess fimbriae, which could help them attach to the supporting material surfaces (Zhang et al., 2011). In this study, *Bacteroidetes* were highly enriched on the membrane surface and enhanced the bacteria adhering to the membrane. It was worth noting that *Bacteroidetes* presented as the dominant members in both MBRs' biofilm sludge samples. Faster membrane fouling occurred in the C-MBR due to the relatively high abundance of *Bacteroidetes* in the bulk sludge. It is illustrated that the high abundance of *Bacteroidetes* in the biofilm sludge from the MAS-MBR may result from the longer duration (48 days) than that of the C-MBR (28 days). It should be noted that *Planctomycetes* were significantly enriched from 8.70% and 8.10% of bulk sludge to 24.10% and 21.40% of biofilm sludge for the C-MBR and MAS-MBR, respectively. The enrichment of *Planctomycetes* in the biofilm sludge may be attributed to its stalk extending from the cell body at the non-reproductive end, which can help it to attach on the membrane surface. Thus it can be inferred that *Planctomycetes* may also play an important role in membrane fouling. *Nitrospirae* were also found to be enriched from 0.36% and 0.30% in bulk sludge to 8.80% and 0.90% in

biofilm sludge for the C-MBR and MAS-MBR, respectively.

At the class level (Figure 3 (b)), 18 bacterial classes present as ≥1% of the sequence reads were detected in all five libraries. The main members affiliated to *Bacteroidetes* were *Sphingobacteriia* (0.78% - 20.11%), *Cytophagia* (1.29% - 16.44%) and *Flavobacteriia* (0.27% - 1.73%). In the *Proteobacteria* phylum, the main class was related to *Betaproteobacteria*, followed by *Alphaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. *Planctomycetacia* was identified as the main subgroup of phyla *Planctomycetes* for all the MBR samples, and it was significantly enriched in the biofilm sludge compared to the bulk sludge in both MBRs. It has been reported that some specific bacteria, specifically *Betaproteobacteria* and *Sphingobacteriia* are the pioneers of surface colonization on membranes (Chen et al., 2015). It can therefore be stated these shared dominant bacteria from bulk sludge and biofilm sludge may be responsible for the initial membrane fouling.

At the genus level, hierarchically clustered heatmap analysis was performed on the top 30 genera (Figure 4). It is obvious that the most representative genera of the biofilm sludge from two MBRs were more similar than their matched bulk sludge, suggesting some specific species were responsible for membrane fouling. The most abundant bacterial genera obtained from biofilm sludge of the C-MBR and MAS-MBR were *Planctomyces* (20.34% and 17.25%), *Cytophagaceae* (9.76% and 15.18%), *env.OPS_17* (3.49% and 7.03%), *Nitrospira* (8.88% and 0.93%), *Thauera* (6.05% and 3.20%), *Terrimonas* (5.36% and 2.68%) and *norank_f_OPB56* (4.13% and 2.43%), respectively. In contrast, the genera obtained from bulk sludge of the C-MBR and MAS-MBR were *Planctomyces* (3.96% and 2.12%), *Cytophagaceae* (15.21% and 8.31%), *env.OPS_17* (0%

and 0%), *Nitrospira* (0.36% and 0.30%), *Thauera* (14.51% and 12.88%), *Terrimonas* (10.92% and 4.09%) and *norank_f_OPB56* (0% and 0%), respectively. It is interesting that all the most abundant genera from biofilm sludge revealed higher abundance in the bulk sludge of the C-MBR than the MAS-MBR except null ones. Demonstrated here is the fact that severe membrane fouling in the C-MBR may be aroused by enriching the pioneer bacteria from bulk sludge. These results suggest that some species only grew on the membrane surfaces, such as the genera *env.OPS_17* and *norank_f_OPB56*. It should be noted that some dominant genera in the bulk sludge derived from the MAS-MBR were in greater abundance than that from the C-MBR, including *Simplicispira* (17.15% and 2.21%), *Aminobacter* (6.06% and 0.76%), *PHOS-HE51* (5.89% and 4.41%), *Pirellula* (5.09% and 4.06%), *unclassified_f_Chitinophagaceae* (3.56% and 3.31%), respectively. The enrichment of these bacteria may be associated with the degradation of large molecules and less membrane fouling in the MAS-MBR.

To make community-wide comparisons between the C-MBR and MAS-MBR, Venn diagrams were plotted to show the shared and unique OTUs (Fig. 5). In the C-MBR, the sum of total observed OTUs in bulk sludge and biofilm sludge was 330, and they shared 217 OTUs. In the MAS-MBR, the sum of total observed OTUs in bulk sludge and biofilm sludge was 319, and they shared 199 OTUs. It can be concluded that some microbes adhered to the membrane surface had originated from the bulk sludge. Furthermore these bacteria are likely to be the pioneer species on the membrane surface, which can lead to severe membrane fouling.

For the bulk sludge, the sum of total observed OTUs from the two MBRs was 289, and they shared 215 OTUs. For the biofilm sludge, the sum of total observed OTUs

from the two MBRs was 334, and they shared 245 OTUs. It can be seen that the microbial community of the biofilm sludge samples from the two MBRs shared more similar features than their matched bulk sludge samples, suggesting that some specific bacteria were selected preferentially on the membrane surfaces. This finding is consistent with the cluster analysis from Heatmap.

The variations on the microbial communities from the two MBRs at the phyla, class and genus levels confirmed that the added magnetic powder significantly affected the microbial community and composition, further altered the microbial metabolites, and consequently affected the membrane fouling evolution. This study: firstly, provides a better understanding of the mechanism of membrane fouling mitigation; and secondly, develops the corresponding control strategy by adding magnetic powder in MBRs.

## 4. Conclusions

Conclusively, this study could provide a promising strategy for mitigating membrane fouling by adding magnetic powder in MBRs from the two main findings: (i) magnetic bio-effect was found to be the main factor to mitigate membrane fouling; and (ii) analyses of bacterial community indicated that severe membrane fouling in the C-MBR may be triggered by enrichment of pioneer bacteria from bulk sludge.

## Acknowledgements

The authors appreciate the financial support of the National Natural Science Foundation of China (51408588 and 21307093), the Youth Innovation Promotion Association, CAS (2017353), the Fund for Science and Technology Development of Pudong (PKJ2014-C12), the State Key Laboratory of Separation Membranes and Membrane Processes (Tianjin Polytechnic University, M2-201605). The authors are also grateful

for the joint collaboration among SARI, Shanghai University, University of Technology Sydney and Tianjin Polytechnic University.

**References**

1. Aslam, M., Charfi, A., Lesage, G., Heran, M., Kim, J., 2017. Membrane bioreactors for wastewater treatment: A review of mechanical cleaning by scouring agents to control membrane fouling. Chem. Eng. J. 307, 897-913.
2. Baygi, A.Z., Moslemi, M., Mirzaei, S.H., 2016. The combination of KMnO4 oxidation and polymeric flocculation for the mitigation of membrane fouling in a membrane bioreactor. Sep. Purif. Technol. 159, 124-134.
3. Chen, C.H., Fu, Y., Gao, D.W., 2015. Membrane biofouling process correlated to the microbial community succession in an A/O MBR. Bioresour. Technol. 197, 185-192.
4. Chen, W., Luo, J., Cao, R., Li, Y., Liu, J., 2017. Effect of macroporous adsorption resin-membrane bioreactor hybrid system against fouling for municipal wastewater treatment. Bioresour. Technol. 224, 112-117.
5. Deng, L., Guo, W., Ngo, H.H., Zhang, H., Wang, J., Li, J., Xia, S., Wu, Y., 2016. Biofouling and control approaches in membrane bioreactors. Bioresour. Technol. 221, 656-665.
6. Deng, L., Guo, W., Ngo, H.H., Zhang, J., Liang, S., Xia, S., Zhang, Z., Li, J., 2014. A comparison study on membrane fouling in a sponge-submerged membrane bioreactor and a conventional membrane bioreactor. Bioresour. Technol. 165, 69-74.
7. Ding, Y., Tian, Y., Li, Z., Zuo, W., Zhang, J., 2015. A comprehensive study into

fouling properties of extracellular polymeric substance (EPS) extracted from bulk sludge and cake sludge in a mesophilic anaerobic membrane bioreactor. Bioresour. Technol. 192, 105-114.

8. Ding, Y., Tian, Y., Liu, J., Li, N., Zhang, J., Zuo, W., Li, Z., 2016. Investigation of microbial structure and composition involved in membrane fouling in the forward osmosis membrane bioreactor treating anaerobic bioreactor effluent. Chem. Eng. J. 286, 198-207.
9. Fykse, E.M., Aarskaug, T., Madslien, E.H., Dybwad, M., 2016. Microbial community structure in a full-scale anaerobic treatment plant during start-up and first year of operation revealed by high-throughput 16S rRNA gene amplicon sequencing. Bioresour. Technol. 222, 380-387.
10. Hu, Y.S., Wang, X.C., Yu, Z.Z., Ngo, H.H., Sun, Q.Y., Zhang, Q.H., 2016. New insight into fouling behavior and foulants accumulation property of cake sludge in a full-scale membrane bioreactor. J. Membr. Sci. 510, 10-17.
11. Gao, D.-W., Zhang, T., Tang, C.-Y.Y., Wu, W.-M., Wong, C.-Y., Lee, Y.H., Yeh, D.H., Criddle, C.S., 2010. Membrane fouling in an anaerobic membrane bioreactor: Differences in relative abundance of bacterial species in the membrane foulant layer and in suspension. J. Membr. Sci. 364 (1-2), 331-338.
12. Guo, X., Miao, Y., Wu, B., Ye, L., Yu, H., Liu, S., Zhang, X.X., 2015. Correlation between microbial community structure and biofouling as determined by analysis of microbial community dynamics. Bioresour. Technol. 197, 99-105.
13. Liu, Y., Li, J.X., Guo, W.S., Ngo, H.H., Hu, J.J., Gao, M.T., 2017. Use of magnetic powder to effectively improve the performance of sequencing batch reactors (SBRs)

in municipal wastewater treatment. Bioresour. Technol. http://dx.doi.org/10.1016/j.biortech.2017.06.069.

14. Ma, B., Wang, S., Li, Z., Gao, M., Li, S., Guo, L., She, Z., Zhao, Y., Zheng, D., Jin, C., Wang, X., Gao, F., 2017. Magnetic $Fe_3O_4$ nanoparticles induced effects on performance and microbial community of activated sludge from a sequencing batch reactor under long-term exposure. Bioresour. Technol. 225, 377-385.
15. Meng, F., Chae, S.R., Drews, A., Kraume, M., Shin, H.S., Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Res. 43(6), 1489-1512.
16. Meng, F., Zhang, S., Oh, Y., Zhou, Z., Shin, H.S., Chae, S.R., 2017. Fouling in membrane bioreactors: An updated review. Water Res. 114, 151-180.
17. Meng, F., Zhou, Z., Ni, B.J., Zheng, X., Huang, G., Jia, X., Li, S., Xiong, Y., Kraume, M., 2011. Characterization of the size-fractionated biomacromolecules: tracking their role and fate in a membrane bioreactor. Water Res. 45(15), 4661-4671.
18. Miao, Y., Guo, X., Jiang, W., Zhang, X.X., Wu, B., 2017. Mechanisms of microbial community structure and biofouling shifts under multivalent cations stress in membrane bioreactors. J. Hazard. Mater. 327, 89-96.
19. Qi, C., Wang, J.N., Lin, Y.H., 2016. New insight into influence of mechanical stirring on membrane fouling of membrane bioreactor: Mixed liquor properties and hydrodynamic conditions. Bioresour Technol. 211, 654-663.
20. Semblante, G.U., Tampubolon, S.D.R., You, S.-J., Lin, Y.-F., Chang, T.-C., Yen, F.-C., 2013. Fouling reduction in membrane reactor through magnetic particles. J.

Membr. Sci. 435, 62-70.

21. Seviour, T., Yuan, Z., van Loosdrecht, M.C., Lin, Y., 2012. Aerobic sludge granulation: a tale of two polysaccharides?. Water Res. 46(15), 4803-4813.
22. Wang, H., Chen, Z., Miao, J., Li, Y., 2016. A novel approach for mitigation of membrane fouling: Concomitant use of flocculant and magnetic powder. Bioresour Technol. 209, 318-325.
23. Xia, S., Li, J., He, S., Xie, K., Wang, X., Zhang, Y., Duan, L., Zhang, Z., 2010. The effect of organic loading on bacterial community composition of membrane biofilms in a submerged polyvinyl chloride membrane bioreactor. Bioresour Technol. 101(17), 6601-6609.
24. Xia, T., Gao, X., Wang, C., Xu, X., Zhu, L., 2016. An enhanced anaerobic membrane bioreactor treating bamboo industry wastewater by bamboo charcoal addition: Performance and microbial community analysis. Bioresour. Technol. 220, 26-33.
25. Yang, X.L., Song, H.L., Lu, J.L., Fu, D.F., Cheng, B., 2010. Influence of diatomite addition on membrane fouling and performance in a submerged membrane bioreactor. Bioresour. Technol. 101(23), 9178-9184.
26. Ye, Y.L., Sailkaly, P.E., Logan, B.E., 2017. Simultaneous nitrogen and organics removal using membrane aeration and effluent ultrafiltration in an anaerobic fluidized membrane bioreactor. Bioresour. Technol. 244, 456-462.
27. Ying, C., Umetsu, K., Ihara, I., Sakai, Y., Yamashiro, T., 2010. Simultaneous removal of organic matter and nitrogen from milking parlor wastewater by a magnetic activated sludge (MAS) process. Bioresour. Technol. 101(12), 4349-4353.

28. Zhang, D., Li, J., Guo, P., Li, P., Suo, Y., Wang, X., Cui, Z., 2011. Dynamic transition of microbial communities in response to acidification in fixed-bed anaerobic baffled reactors (FABR) of two different flow directions. Bioresour. Technol. 102(7), 4703-4711.

29. Zhou, L., Huang, B., Huang, D., Niu, C., Zeng, G., Ruan, M., Zhang, X., 2015. A feasible strategy for promoting activated sludge hydrolysis by using ironporphyrin modified $Fe_3O_4$ nanoparticles as an efficient biomimic catalyst. Chem. Eng. J. 280, 248-255.

30. Zhou, L., Zhang, Z., Xia, S., Jiang, W., Ye, B., Xu, X., Gu, Z., Guo, W., Ngo, H.H., Meng, X., Fan, J., Zhao, J., 2014. Effects of suspended titanium dioxide nanoparticles on cake layer formation in submerged membrane bioreactor. Bioresour. Technol. 152, 101-106.

**Figure captions**

Figure 1: The compositions of SMP and EPS of bulk sludge in both MBRs.

Figure 2: MW distributions of SMP, EPS and MBR permeate in the two MBRs (a) the C-MBR and (b) the MAS-MBR.

Figure 3: Taxonomic classification of the microbial community at phylum and class level for each sample, present as $\geqslant$1% of the sequence reads in at least one sample.

Figure 4: Heatmaps showed the relative abundance of the top 30 species at genus level for each sample.

Figure 5: Venn diagrams at OTU level of bulk sludge and biofilm sludge in the C-MBR and MAS-MBR.

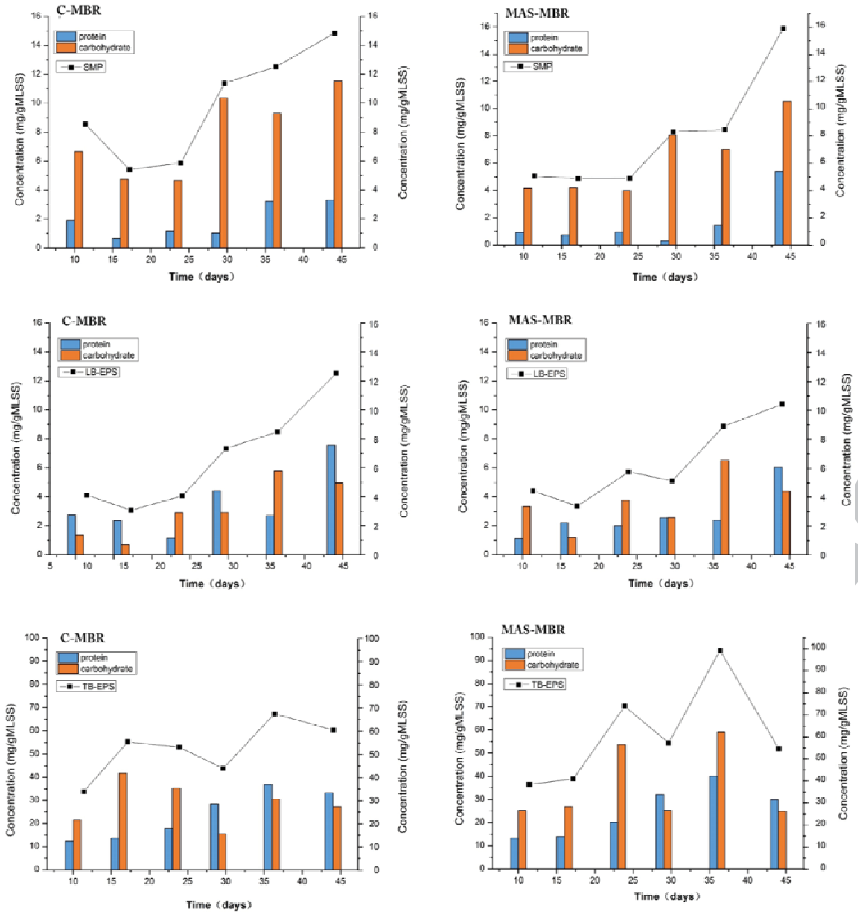



Figure 1

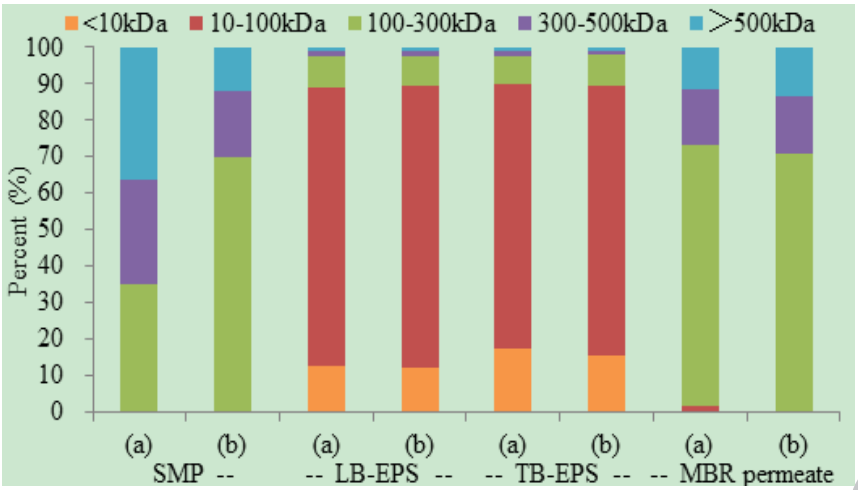



Figure 2

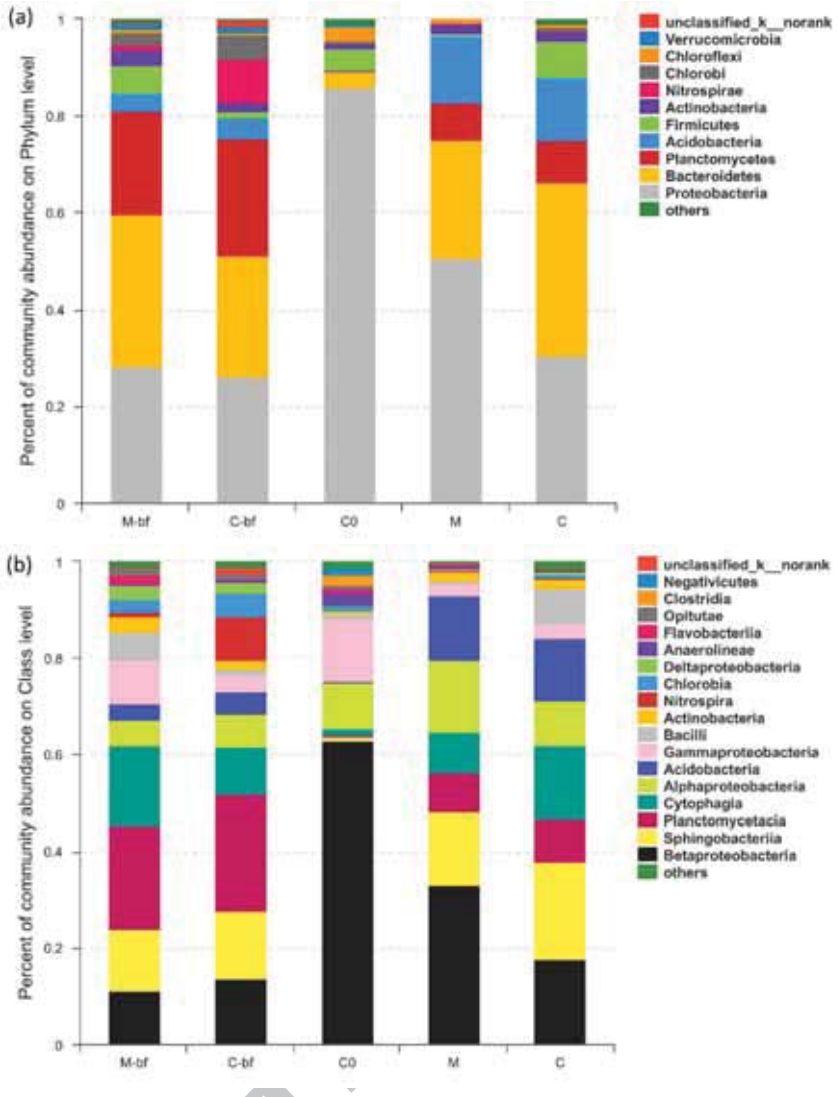

(a)
Percent of community abundance on Phylum level
0
0.2
0.4
0.6
0.8
1
M-bf
C-bf
C0
M
C
unclassified_k__norank
Verrucomicrobia
Chloroflexi
Chlorobi
Nitrospirae
Actinobacteria
Firmicutes
Acidobacteria
Planctomycetes
Bacteroidetes
Proteobacteria
others
(b)
Percent of community abundance on Class level
0
0.2
0.4
0.6
0.8
1
M-bf
C-bf
C0
M
C
unclassified_k__norank
Negativicutes
Clostridia
Opitutae
Flavobacteriia
Anaerolineae
Deltaproteobacteria
Chlorobia
Nitrospira
Actinobacteria
Bacilli
Gammaproteobacteria
Acidobacteria
Alphaproteobacteria
Cytophagia
Planctomycetacia
Sphingobacteriia
Betaproteobacteria
others

Figure 3

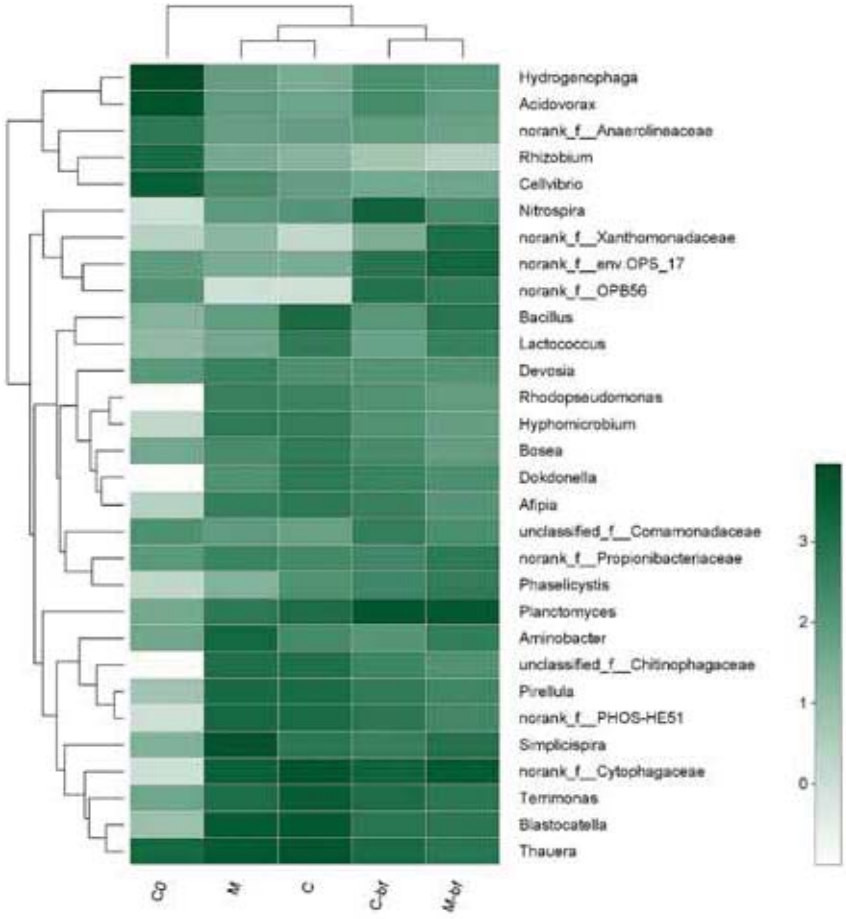



Figure 4

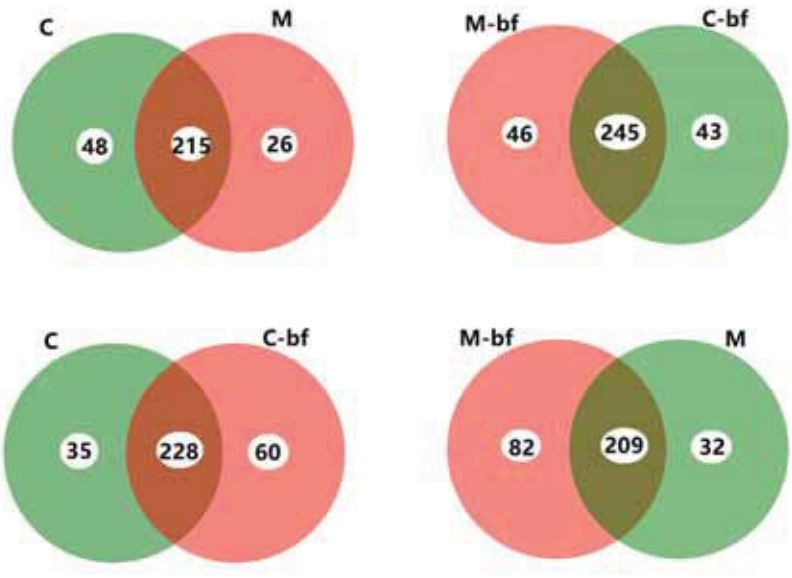



Figure 5

**Table captions**

Table 1: Feed wastewater and operating conditions of both MBRs.

Table 2: Comparison of COD and $NH_4^+$-N removals in both MBRs.

Table 3: Richness and diversity estimators of the bacterial phylotypes of bulk sludge and biofilm sludge in both MBRs.

Table 1

| Operational parameters | C-MBR | MAS-MBR |
|---|---|---|
| Feed COD(mg/L) | 309.51±79.70 | 309.51±79.70 |
| Feed $NH_4^+$-N(mg/L) | 58.98±15.24 | 58.98±15.24 |
| DO in the MBR tank(mg/L) | 7.41±0.63 | 7.45±0.62 |
| pH in the MBR tank(mg/L) | 7.61±0.60 | 7.66±0.69 |
| HRT(h) | 8 | 8 |
| SRT | No waste | No waste |
| Aeration intensity($m^3$/h) | 0.1 | 0.1 |

[a] Numbers are means ± standard deviations

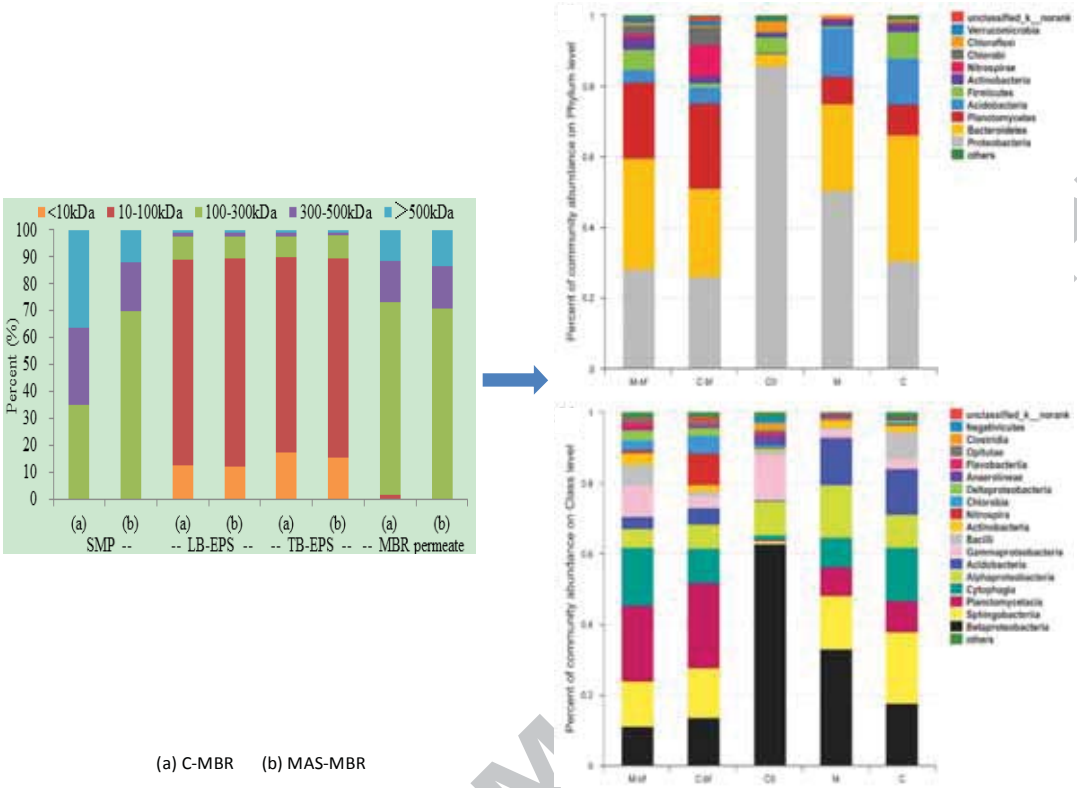
Table 2

| Parameters | C-MBR | MAS-MBR |
|---|---|---|
| Effluent COD | 33.59±6.53 | 30.51±6.65 |
| Effluent $NH_4^+$-N | 2.78±2.67 | 1.95±1.68 |
| COD removal efficiency (%) | 86.40±5.58 | 88.75±4.88 |
| $NH_4^+$-N removal efficiency (%) | 90.78±15.32 | 92.74±12.40 |

Table 3

| Samples | Optimized sequences | Average Length | OTUs | Chao1 index | Ace index | Shannon index | Simpson index | Coverage index |
|---|---|---|---|---|---|---|---|---|
| C0 | 34817 | 395 | 216 | 257.0 | 247.9 | 2.56 | 0.1705 | 0.998501 |
| C | 43838 | 395 | 263 | 284.3 | 289.2 | 3.31 | 0.0731 | 0.998895 |
| M | 38133 | 395 | 241 | 268.8 | 274.9 | 3.27 | 0.0725 | 0.998634 |
| C-bf | 34552 | 395 | 288 | 311.3 | 309.6 | 3.69 | 0.0555 | 0.998590 |
| M-bf | 31777 | 395 | 291 | 302.8 | 306.8 | 3.72 | 0.0599 | 0.998927 |

## GRAPHICAL ABSTRACT




(a) C-MBR (b) MAS-MBR