



## Effect of different types of fillers in membrane bioreactors for greywater treatment and membrane fouling

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### ABSTRACT

This study investigated the effects of different types of fillers fed into membrane bioreactors (MBR) in greywater treatment for water purification effect, membrane fouling and microbial community structure. Two MBR systems of hollow fiber membrane and flat sheet organic membrane were running with floating sponge filler and fixing combination filler. The MBR systems with filler improved the removal rate of ammonia nitrogen, total nitrogen, and total phosphorus compared with that without filler, especially the effect of floating sponge filler was obvious (91.49%, 71.53%, and 72.35% in the flat sheet membrane system). The floating sponge filler also effectively improved the permeation performance of the MBR system, but the fixed type filler (combination filler) accelerated membrane fouling and reduced the permeation performance of the system. This finding may be due to the higher abundance of Gammaproteobacteria (relative abundance 38.3%) and Bacteroidia (relative abundance 8.35%) on the filler surface. Feeding fillers into MBR can improve the nitrogen removal capacity, effluent quality, and permeability of the system. However, it depends on the type and material of different fillers.

*Keywords:* Membrane bioreactor; Biological fillers; Membrane fouling; Greywater

### 1. Introduction

Domestic wastewater comes from washing, showering, laundry, toilet flushing, and kitchen water, and can be divided into two categories, black water and greywater, in accordance with the source. Greywater refers to domestic sewage, including washing, shower, laundry, and kitchen water, excluding toilet feces and toilet flushing mixed wastewater [1]. Greywater can be divided into light greywater and black greywater. Light greywater includes wastewater from bathtubs, showers, and hand washing, and black greywater

includes wastewater from kitchen sinks, dishwashers, laundry rooms, and washing machines [2]. The total greywater includes the above two types of greywater, also known as mixed greywater [3]. Greywater is generated by the living habits, products used, and drainage methods of relevant personnel, so its characteristics are highly variable. Compared to the (COD – chemical oxygen demand) COD:N:P ratio of domestic sewage, light greywater has lower nitrogen and phosphorus content, while black greywater contains kitchen sewage, which is similar to ordinary domestic sewage. Although there are variations in greywater quality, all

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types of greywater show good biodegradability in terms of the COD:BOD<sub>5</sub> (BOD<sub>5</sub> – biochemical oxygen demand) ratios [4]. Studies have shown that the ratio of BOD<sub>5</sub>:COD in greywater is more than 0.30 which indicates good potential for biological treatment [5,6]. The concentrations of nutrients also show no apparent limitation for the growth of microorganisms. The study of Palmquist and Hanaeus [7] found that greywater is high in S, Ca, K and Al and the concentration levels of the trace nutrients met the growth of microorganisms. The amount of greywater generated in households usually accounts for 50% to 80% of the total domestic wastewater [8]. If dry toilets, vacuum toilets, and negative pressure drainage systems are used, then the percentage of greywater can increase to 90% of the total domestic wastewater [9]. Among domestic wastewater, greywater is the most promising source of reuse water because it is produced in the largest quantity, has low pollution level, and has the characteristics of simple reuse treatment process and low cost. Among the existing grey water treatment, membrane bioreactor (MBR) has the advantages of good effluent quality, small sludge production, small footprint, and stable effluent to meet regeneration and reuse standards [10,11].

In recent years, MBR have been increasingly used in wastewater treatment because the complete separation of sludge retention time (SRT) and hydraulic retention time (HRT) makes the reactor have a great purification capacity and shock load resistance [12,13]. With the in-depth research on membrane materials and membrane production process, the manufacturing cost of membrane modules is gradually decreasing. However, membrane fouling is a great obstacle to the large-scale application of MBR [14]. With the continuous operation of MBR, the activated sludge in the reactor is in contact with the membrane module for a long time, and some soluble substances and small particles in it penetrate into the surface and inside of the membrane, resulting in the degradation of the filtration performance of MBR. Membrane fouling can affect effluent quality and cause frequent cleaning of the membrane modules, shortening

their service life and increasing the construction and operation costs of the treatment system. Membrane fouling is unavoidable, and MBR research focuses on how to delay the time of membrane fouling and extend the life of membrane modules. In addition to selecting membrane materials with high resistance to fouling, changing the activated sludge characteristics and operating conditions of the reactor is an effective means to suppress membrane fouling [10]. Several researches have shown that the addition of fillers to a conventional MBR can help the operation of the reactor in various ways, including improved pollutant removal, reduction of the concentration of suspended solids, improved filterability, and reduction of membrane cake layer formation by scouring effects of the suspended fillers [15–18].

Currently, limited studies are reported on the effects of different types of fillers on treatment performance and membrane fouling in the case of MBR treatment of greywater. To this end, experiments were conducted to analyze the effect of MBR on the removal of pollutants, such as COD, NH<sub>3</sub>-H, total nitrogen (TN), and total phosphorus (TP), and the effect of changes in soluble microbial products (SMP) and extracellular polymeric substances (EPS) in the reactor on membrane fouling by feeding different types of biological fillers under the operating conditions of intermittent aeration. The effect of microbial community on membrane fouling was explored by microbial community analysis based on high-throughput 16S rRNA gene sequencing. The aim was to optimize the operation effect of MBR while providing reference for the large-scale application of MBR in greywater treatment.

## 2. Materials and methods

### 2.1. Experiment materials and operation

Fig. 1 demonstrates the installation of the MBR system. Two types of membranes, namely, hollow polyvinylidene fluoride (PVDF) fiber membrane (KAIMI, Jiangsu Province,

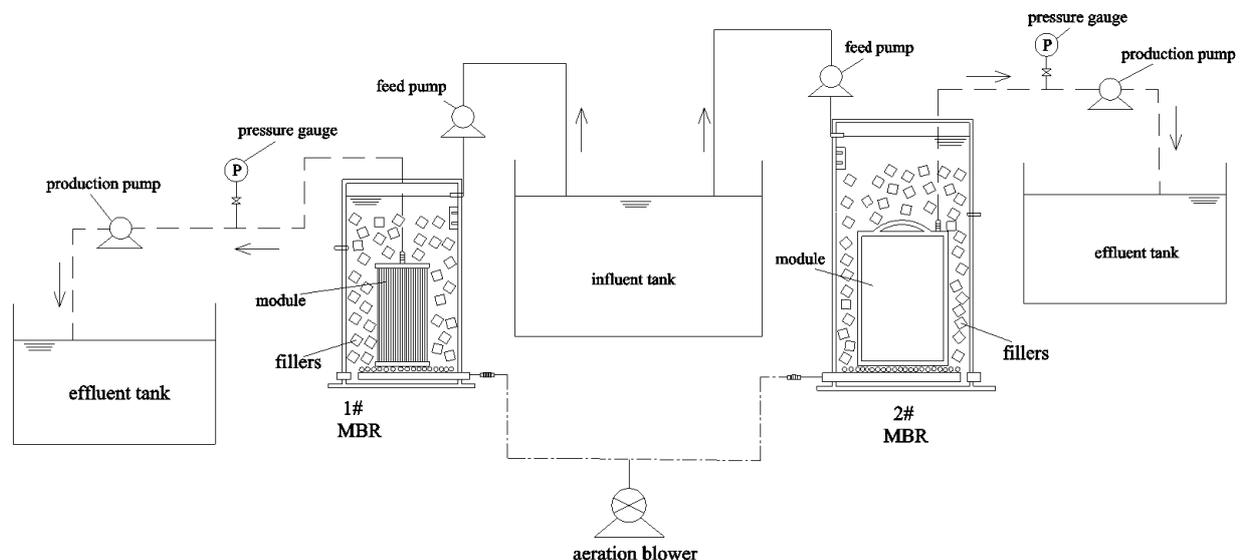


Fig. 1. Schematic diagram of the hollow fiber MBR (#1MBR) and flat sheet organic MBR (#2MBR) systems.

China) and flat PVDF organic membrane (PEIER, Jiangsu Province, China), were used in this experiment. The two membranes were externally pressed with a nonwoven polyethylene terephthalate liner, with a 0.1-micron membrane pore size and excellent hydrophobic properties. The total effective filtration area of hollow fiber membrane module was 0.063 m<sup>2</sup>, and that of flat sheet membrane module was 0.1008 m<sup>2</sup>. The experiment has two sets of equipment, namely, #1MBR using hollow fiber membrane and #2MBR using flat sheet membrane. The total effective volume of #1MBR is 12 L, with a size of 20 cm × 10 cm × 62.5 cm, and the effective water depth in the reactor is 60 cm. The total effective volume of #2MBR is 21 L, with a size of 30 cm × 12 cm × 60 cm, and the effective water depth in the reactor is 58.3 cm. The two reactors are made of acrylic plate. The reactor was filled with floating sponge filler and combination filler (Fig. S1), and the filling ratio was 20%. The floating sponge filler is a 2 cm square sponge made of polyurethane. The combination filler consists of a central rope and several plastic sheets with fiber bundles, made of high-density polyethylene. Dissolved oxygen (DO) in the reactor was controlled at 3–5 mg/L through blast aeration. This process provides a good environment for the growth of microorganisms.

The activated sludge was taken from the return sludge of the secondary setting tank of Qiaobei Wastewater Treatment Plant in Nanjing, China. The activated sludge was incubated and domesticated for 2 weeks before being placed in the reactors. Synthetic greywater was fed to the reactors consisted of kitchen and detergent wastewater, NH<sub>4</sub>Cl, and KH<sub>2</sub>PO<sub>4</sub>. The content of mineral solution was as described by Yang et al. [19], MgSO<sub>4</sub>·7H<sub>2</sub>O (25 mg/L), FeSO<sub>4</sub>·2H<sub>2</sub>O (20 mg/L), and CaCl<sub>2</sub>·2H<sub>2</sub>O (22 mg/L). The reactor influent contained 250–350 mg·COD/L, 25–30 mg·NH<sub>3</sub>-N/L, 30–35 mg·TN/L, and 3–3.5 mg·TP/L. The pH in the reactors was maintained at 7.0–8.0 by NaHCO<sub>3</sub>.

The influent tank was connected to each reactor, and the greywater entered the reactor through the pumping action of a peristaltic pump (Kamoer, Shanghai, China). In each reactor, the membrane module was connected to a magnetic drive gear pump (Ouruik, Nanjing, China), and the effluent water was achieved by the suction force generated by the pump. Transmembrane pressure (TMP) was measured periodically by the digital pressure gauge (Asmik, Hangzhou, China). The reactor adopted an intermittent aeration mode with an optimal aeration stop ratio of 15/5 min, and the MBR systems operated continuously for 8 h/d. When the reactor in operation for a period of time, the membrane modules were removed and cleaned by immersion in 0.1% NaClO

solution. The initial mixed liquid suspended solids (MLSS) concentration of the reactor was 3,500 mg/L, and the MLSS concentration increased under different operating conditions. When the reactor was operated steadily under different operating conditions, the effluent was sampled at the end of each day of operation, and the concentrations of COD, NH<sub>3</sub>-N, TN, and TP in the effluent were recorded.

## 2.2. Experimental design

In this study, floating sponge filler and fixing combination filler were fed into the reactor to treat the experimental greywater under optimal aeration conditions. The operating conditions of each reactor are shown in Table 1. Each reactor was operated under optimal operating conditions for a period of time as a control experiment before the filler was fed. The reactor was running normally without filler in the first stage, the floating sponge filler was added in the second stage, and the filler was combination filler in the third stage.

## 2.3. Analysis methods

### 2.3.1. Analysis of water quality

The measurement methods used in this study were the standard method recommended by the Ministry of Ecology and Environment of the People's Republic of China [20]. The COD, TN, and TP of water sample were determined by using a spectrophotometer (DR2800, HACH, USA) after rapid digestion (DRB200, HACH, USA). NH<sub>3</sub>-N was measured by using an ultraviolet (UV) spectrophotometer (T6 New Century, Beijing Purkinje Instrument, China). MLSS and SS concentrations were measured by using an analytical balance (FA2004, Tianjin Balance Instrument, China). The DO content in the reactor was measured by using an electrochemical probe (MTC101, HACH, USA). The pH was recorded by using a pH analyzer (pHC101, HACH, USA).

### 2.3.2. Analysis of sludge characteristics

The main substances causing membrane fouling in MBR are EPS and SMP. In this study, water-soluble EPS in activated sludge was obtained by using a cation exchange resin [21]. A 10 mL activated sludge sample was taken from the #1MBR and #2MBR by using a 50 mL centrifuge tube. The sample was then placed in a centrifuge (TGL-16m, Xiangyi, China) and centrifuged at 4°C and 3,000 g (RCF) for 10 min. The supernatant was removed, and 5 mL PBS solution was

Table 1  
Operating conditions

Factor	#1MBR			#2MBR		
	No filler	Floating filler	Combination filler	No filler	Floating filler	Combination filler
Aeration rate	3 L/min			5 L/min		
Aeration stop ratio (min/min)	15:15			15:10		
Effluent time (h/d)	8	8	8	8	8	8
Period (d)	12	12	12	12	12	12
Activated sludge (mg/L)	3,500–6,000			4,500–7,000		

added. The centrifuge tube was shaken in a vortex shaker (Vortex Genius 3, IKA, China) for 1 min, and centrifuged at 4°C and 3,000 g for 10 min. Removed 5 mL of supernatant, added 5 mL of PBS solution and repeated the above steps 2–3 times. The centrifuged sludge was removed and recalculated at 50 mL. An amount of cation exchange resin (65 g resin/g MLSS) was added to a 100 mL triangular flask together with the mud-water mixture and shaken in a constant temperature shaker (ZQPZ-115, LEIBO TERRY, China) at 4°C for 12 h at a speed of 200 rpm. The supernatant was filtered through a 0.45 µm membrane. The filtration fluid was centrifuged (4°C, 11,000 g) for 0.5 h, and the supernatant was removed. The procedure was repeated twice, and the rest was extracted EPS. The SMP extraction from activated sludge was achieved by using a high-speed centrifugal method [22]. The SMP was removed from the sludge by centrifugation at 5,000 g for 10 min and filtered through a 0.22 µm filter membrane. The filtration fluid, which contained the SMP, was extracted and stored in a refrigerator for later analysis. The main components in EPS and SMP were proteins and polysaccharides [23–25]. The concentration of polysaccharides was determined by anthracene-sulfur method [26]. Protein concentrations were determined with a modified Lowry Protein Assay Kit (Sangon Biotech, Shanghai, China) via spectrophotometry. Bovine serum protein and glucose were used as the standard reference for protein and polysaccharides.

### 2.3.3. Analysis of membrane fouling

In this study, the degree of membrane fouling was determined by TMP. With the increase in the TMP, the greater the resistance of membrane filtration, the lower the membrane permeation performance, and the lower the effluent rate of the membrane module, resulting in lower treatment efficiency of the MBR system. The MBR flux was calculated as the volume of effluent collected from each system divided by the area of the membrane and the filtration period, as shown in Eq. (1).

$$J = \frac{V}{A \cdot T} \quad (1)$$

where  $V$  = cumulative filtering volume in a filtration period (L);  $A$  = total membrane area (m<sup>2</sup>);  $T$  = filtration period (h). On the basis of flux and TMP across the membrane, Eq. (2) is used to evaluate the membrane permeability characteristics in different operating conditions.

$$L_p = \frac{J}{\Delta p} \quad (2)$$

where  $J$  is the 6 min flux of the filtration (L/m<sup>2</sup>·h), and  $\Delta p$  is the amount of change in TMP (KPa), which corresponds to every 6 min of operation.

The total membrane resistance ( $R_t$ ) consists of membrane inherent resistance ( $R_m$ ), filter cake layer resistance ( $R_c$ ), and pore blocking resistance ( $R_p$ ), as shown in Eq. (3).  $R_m$  can be measured by the clean membrane under the condition of clean water.  $R_p$  can be obtained from the membrane resistance ( $R_1$ ) measured by the membrane cleaned with 1% NaClO solution in clear water minus the inherent

membrane resistance ( $R_m$ ), and  $R_c$  can be obtained from the total membrane resistance ( $R_t$ ) minus  $R_1$ .

$$R_t = R_m + R_c + R_p \quad (3)$$

The morphology and major elements of the filter cake from fouled membranes were measured with a ZEISS Sigma-300 scanning electron microscope combined with Fourier-transform infrared (FTIR) spectrometer (Bio-rad FTS6000, Thermofisher, USA).

### 2.3.4. Analysis of the microbial composition

Microbial sequencing was performed on the sludge mixture, floating filler and combination filler surface of hollow and flat membrane systems by using 16SRNA high throughput sequencing technology. The main processes of sequencing include: DNA extraction, select primers, PCR amplification, Illumina sequencing, Processing of sequencing data (Fig. S2).

In accordance with the manufacturer instructions, DNA was extracted from the above samples by using an E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.). DNA extracts were examined on a 1% agarose gel, and DNA concentration and purity were determined by using a NanoDrop 2000 UV-visible spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V1–V3 of the bacterial 16S rRNA gene was amplified with primer pairs 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 533R (5'-TTACCGCGGCTG-GCA-3') on an ABI GeneAmp® 9700 polymerase chain reaction (PCR) thermocycler [27]. The PCR amplification of the 16S rRNA gene proceeded as follows: initial denaturation at 95°C for 3 min, followed by denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s, and a single extension at 72°C for 10 min, ending at 10°C. The PCR mixture contained 4 µL of 5× TransStart FastPfu buffer, 2 µL of 2.5 mM dNTPs, forward primer (5 µM) 0.8 µL, reverse primer (5 µM) 0.8 µL, TransStart FastPfu DNA polymerase 0.4 µL, template DNA 10 ng, and 20 µL ddH<sub>2</sub>O was added to. PCR reactions were performed in triplicate. PCR products were extracted from 2% agarose gels and purified by using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the manufacturer instructions and quantified by using a Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar fashion and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) in accordance with the standard protocol of Majorbio Bio-Pharm Technology Co., Ltd., (Majorbio, Shanghai, China).

## 3. Results and discussion

### 3.1. Performance of filler MBR system

The influent and effluent water quality of each system was measured daily to determine the changes in COD, NH<sub>3</sub>-N, TN, and TP in the influent and effluent water, and to calculate the removal efficiency of each system on the above water quality characteristics. This process was performed to study the effects of different fillers on the effluent water quality of MBR system. The results are shown in Fig. 2.

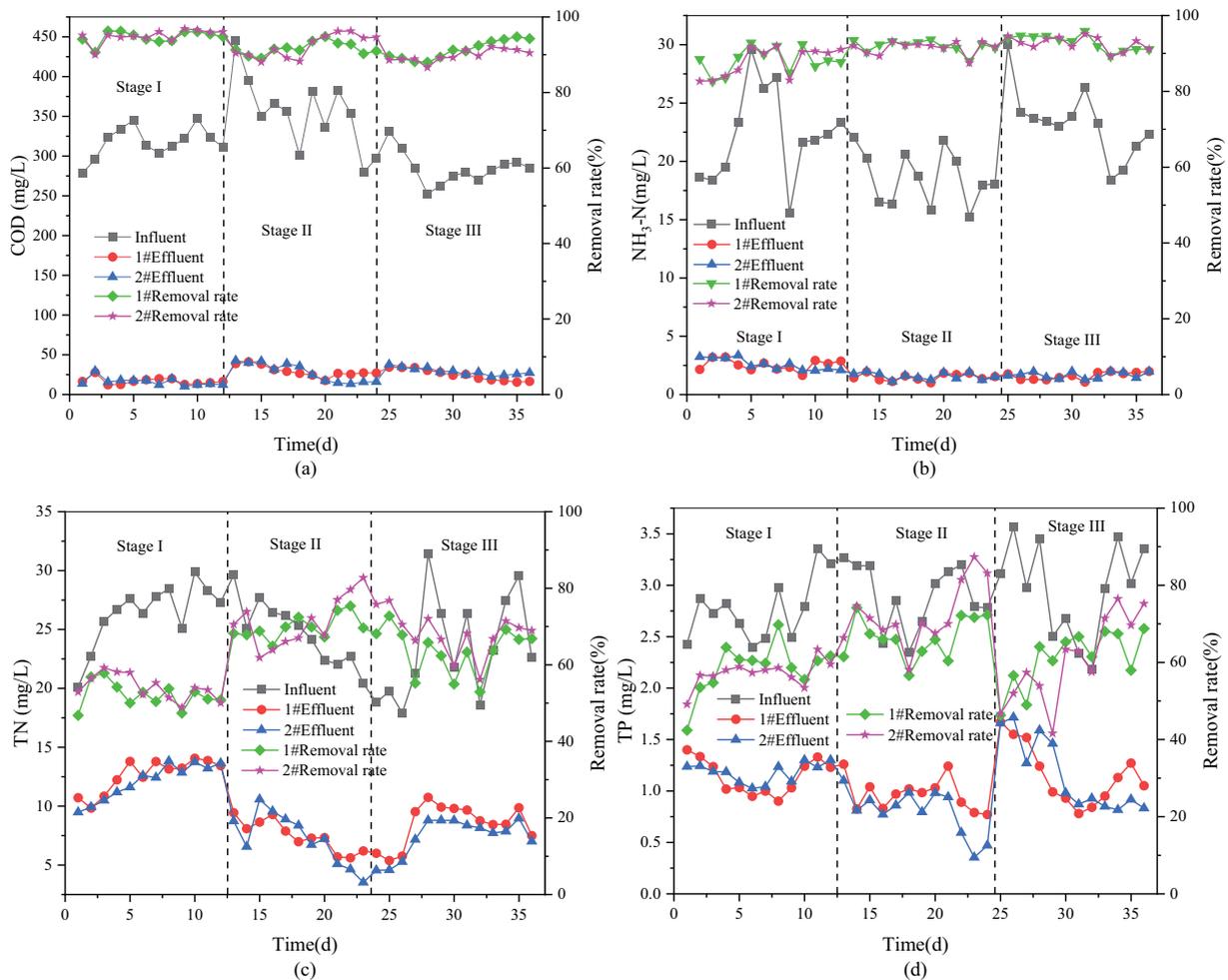


Fig. 2. Variation of chemical oxygen demand (a),  $\text{NH}_3\text{-N}$  (b), total nitrogen (c), total phosphorus (d) and removal efficiency of influent and effluent of hollow fiber MBR (#1MBR) and flat sheet MBR (#2MBR) systems.

Table 2  
Distribution of membrane resistance for #1MBR and #2MBR with different fillers

Membrane resistance distribution	#1MBR			#2MBR		
	No filler	Floating filler	Combination filler	No filler	Floating filler	Combination filler
$R_m/R_t$	12.2%	14.5%	12.7%	3.5%	3.8%	2.3%
$R_p/R_t$	6.6%	13.0%	13.0%	4.7%	2.4%	2.4%
$R_c/R_t$	81.2%	72.4%	74.3%	91.8%	93.8%	95.3%
$R_t$ ( $\times 10^{12} \text{ m}^{-1}$ )	15.2	9.9	16.7	10.8	9.1	14.8

### 3.1.1. Effect of COD removal

As shown in Fig. 2a, the MBR system had good removal of COD in the floating sponge filler and combination filler MBR systems, but they were all lower than the system without filler. The influent COD concentrations of the two systems ranged from 309 to 378 mg/L during the operation stage of the floating sponge filler (stage II). The average COD removal rates of the hollow fiber membrane system and flat sheet membrane system were 92.1% (Table 2) and

90.5%, respectively, which were lower than 94.69% in stage I (no filler). The average effluent concentrations of the hollow fiber membrane system and the flat sheet membrane system were 29.35 and 27.53 mg/L, respectively. When the MBR system was fed with combination filler (stage III), the influent COD concentrations ranged from 252.5 to 330.6 mg/L. The average COD removal rate of the hollow fiber membrane system was 91.3% with an effluent concentration of 24.75 mg/L, and the flat sheet membrane systems was 89.8% with an effluent concentration of 28.95 mg/L.

The average COD removal rate was lower than the MBR system without filler (stage I). This finding may be because most of the microorganisms in the reactors were dormant or semidormant due to the lack of organic carbon source [28]. The original volumetric loading can no longer meet the normal metabolism of microorganisms because the total biomass in the reactors was increased by adding fillers [29]. Therefore, the filler feeding did not have much effect on the COD removal in this MBR system [30], and the COD concentration of the effluent was maintained at a stable level.

### 3.1.2. Effect of nitrogen removal

Nitrogen removal in MBR relies on autotrophic nitrification and heterogeneous denitrification by microorganisms in activated sludge. However, we consider microbial nitrification as an aerobic process and denitrification as an anoxic process. If the level of DO in the reactor is high, then it will depress the denitrification of microorganisms. The addition of fillers as biocarriers in MBR has the potential to reduce membrane fouling, facilitates simultaneous nitrification and denitrification, and promotes nitrogen removal at low influent COD/N ratios due to the aerobic and anoxic environment it creates [31].

As shown in Fig. 2b, the concentration of  $\text{NH}_3\text{-N}$  in the effluent of the MBR system was always maintained at a low level, indicating that the reactor has an extremely excellent ability to remove  $\text{NH}_3\text{-N}$ . The aeration pattern of intermittent aeration did not affect the aerobic nitrification of the reactor. Although the concentration of  $\text{NH}_3\text{-N}$  in the influent water fluctuated sharply, the  $\text{NH}_3\text{-H}$  concentrations in the effluent water of the two MBR systems with floating sponge and combination fillers were maintained at 1.50 and 1.57 mg/L (in #1MBR, removal rate were 91.94% and 92.90%), and 1.61 and 1.67 mg/L (in #2MBR, removal rate were 91.49% and 92.66%), which were lower than 2.54 mg/L (in #1MBR) and 2.60 mg/L (in #2MBR) of the MBR systems without fillers. The results showed that the addition of filler to the MBR can improve the nitrogen removal capacity and efficiency of the MBR system. This result is in good agreement with previous experimental investigations that reported biofilms with high nitrifying activity [32]. In Fig. 2c, the removal of TN differs in different stages and in different reactors. In the hollow fiber membrane system (#1MBR), the TN removal rates of the three stages were 52.06%, 69.71% and 63.47%. In the flat sheet membrane system (#2MBR), the TN removal rates of the three stages were 54.16%, 71.53%, and 60.67%. As a whole, the filler feeding improved the removal efficiency of TN, but the enhancement effect of floating sponge filler was more obvious, and the removal rate increased by 17.5% compared to that without filler. This finding may be due to the structure of the sponge that provides a good anoxic environment on the filler surface and anaerobic conditions inside the sponge. The hollow and porous structure inside the floating sponge filler exposed the biofilm to oxygen permeation smaller in size, and its inner anoxic zone was well retained [33]. The anaerobic–aerobic stratified structure is similar to aerobic granular sludge, and it is conducive to simultaneous nitrification denitrification (SND) of nitrogen [34]. In stage III, the effluent TN concentration of the flat sheet

membrane (#1MBR) was consistently better than that of the hollow fiber membrane (#2MBR), with a 6.8% higher removal rate. This finding shows that the combination filler is more effective for flat membrane than hollow fiber membrane.

### 3.1.3. Effect of phosphorus removal

The removal of phosphorus was measured in terms of TP. As shown in Fig. 2d, the removal effects of the two MBR systems were improved to different degrees after feeding the filler, especially the floating sponge filler was extremely effective. The TP removal rate of #1MBR increased from 58.46% to 66.41%, and #2MBR increased from 57.12% to 72.35%. The combination filler provided some enhancement to the two MBR systems, but not as significant as the floating sponge filler. In stage II, the TP concentration of the effluent of the flat sheet membrane was lower than that of the hollow fiber membrane, indicating that the floating sponge filler was more effective for flat sheet MBR.

The removal of phosphorus in the reactor was performed by polyphosphate-accumulating organisms (PAOs) and went through aerobic and anoxic stages. These organisms can anaerobically store readily biodegradable organic matter as intracellular poly  $\beta$ -hydroxyalkanoates (PHAs), most commonly in the form of polyhydroxybutyrate [35,36] and release orthophosphate into the environment at the same time. During the aerobic phase, PAOs oxidize intracellularly stored PHA as energy and take up orthophosphate [37]. The uptake of orthophosphate in the aerobic stage is greater than the release of orthophosphate in the anoxic stage, thereby achieving a decrease in the orthophosphate content in the effluent. The alternating anoxic–aerobic environment in the reactor facilitates the removal of phosphorus. The alternating anoxic–aerobic environment in the reactor is conducive to phosphorus removal, and the aerobic–anoxic coexisting environment created by the filler feeding has a beneficial effect on the removal of phosphorus.

### 3.2. Influence of membrane permeability

The reactors were operated for 8 h each per day for 12 d. During the operating time, the fluxes of the hollow fiber and flat sheet membrane systems varied consistently, decreased with the increase in operating time but recovered to a high level on the next day. This finding may be because when the water production in the system stops, the aeration system continues to provide aeration to the reactor to maintain the DO concentration required by the activated sludge, and that the turbulence of water flow and bubble scouring during aeration of the reactor removes part of the filter cake layer after the filler was fed. Therefore, aeration in the reactor was used to provide DO while removing a portion of the filter cake layer from the membrane surface to delay membrane fouling when using the MBR to treat decentralized wastewater.

As shown in Fig. 3a, the best permeation performance of the hollow fiber membrane system occurred when the floating sponge filler was fed. The permeation performance at the end of each cycle ranged within  $1.0 \pm 0.2 \text{ L}/(\text{m}^2\cdot\text{h}\cdot\text{KPa})$ . At the beginning of the next cycle, the system permeability returned to within  $2.3 \pm 0.6 \text{ L}/(\text{m}^2\cdot\text{h}\cdot\text{KPa})$ . The permeation

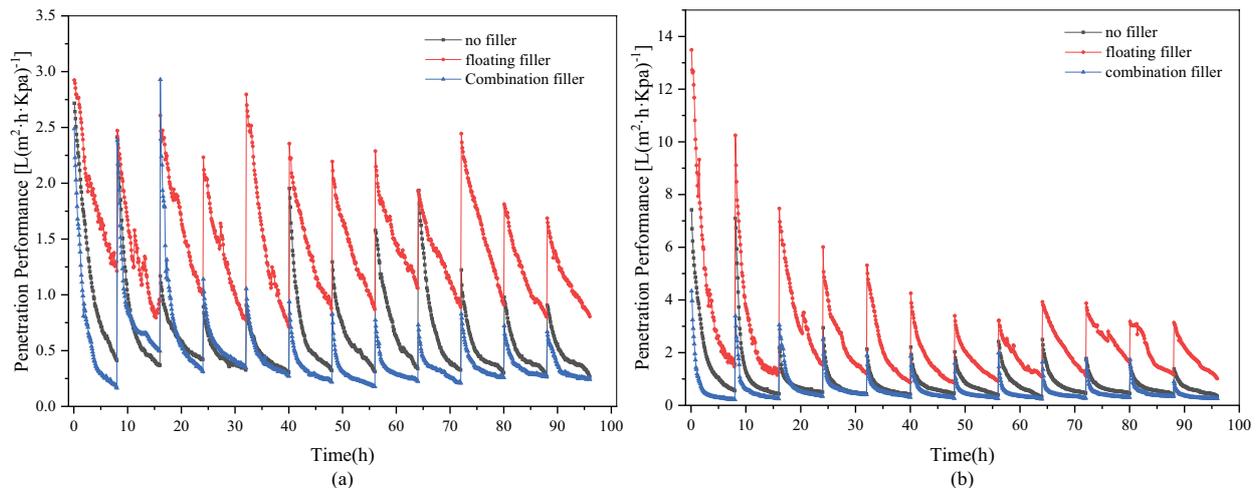


Fig. 3. Comparison of membrane permeability between hollow fiber membranes and flat sheet membranes with different fillers, (a) hollow fiber membranes and (b) flat sheet membranes.

performance of the system with the combination filler was similar to without filler. The permeation performance of the system decreased rapidly during one cycle, ending with only  $0.25 \pm 0.1$  L/(m<sup>2</sup>·h·KPa). The main reasons are as follows: The floating sponge filler material is softer, smaller volume can be evenly distributed in the membrane pool, and the floating sponge filler can form a fluidized state to flush the membrane surface in the aeration process [33], thereby delaying the membrane pollution. In the reactor filled with combination filler, the huge and complex structure of the combination filler inhibits the scouring effect of water flow and bubbles on the membrane surface, which reduces the mobility energy of activated sludge in the reactor and increases the rate of membrane fouling. Similar to the hollow fiber membrane system, the permeability performance of the flat sheet membrane system (Fig. 3b) was higher during operation with floating sponge filler than without filler and lower during operation with combination filler than without filler. The permeation performance of the flat sheet membrane system decreased more rapidly when the floating sponge filler was started to be added, and the permeation performance of the system decreased from 13.49 L/(m<sup>2</sup>·h·KPa) at the beginning to 1.47 L/(m<sup>2</sup>·h·KPa) at the end, a reduction of 89.1%. The beginning permeability performance of each cycle decreased with the increase in running time, and the beginning permeability performance of the last cycle decreased by 76.8% compared with that of the first cycle. This finding is most likely due to the formation of a tight layer of biofilter cake on the membrane surface, and particulate matter from the sludge mixture enters the membrane inside, leading to irreversible fouling [14]. In conclusion, the average permeation performance of the membrane was greatly enhanced by the feeding of floating sponge filler, (124.5% increase in #1MBR, 175.0% increase in #2MBR).

### 3.3. Characteristics of activated sludge mixture

The sludge concentration of the hollow fiber membrane and the flat sheet membrane increased after the feeding of different fillers (Fig. S3), indicating that the

microorganism growth environment was more suitable for growth in this system, and the sludge volume maintained at 70 mL/g, indicating that the sludge settling performance was good. The variation of EPS and SMP concentrations in #1MBR and #2MBR under the feeding of filler is shown in Fig. 4. The main components of EPS and SMP are proteins and polysaccharides [38,39]. Their concentrations were measured on day 12 after the filler was fed and on the basis of their TOC content as a reference [40].

As shown in Fig. 4, the main component of EPS is protein (Fig. 4a and c,  $73.71\% \pm 3.10\%$ ), and that of SMP is polysaccharide (Fig. 4b and d,  $90.68\% \pm 5.70\%$ ). The concentration of EPS ( $25.06 \pm 3.71$  mg/g MLSS) is higher than the concentration of SMP ( $3.06 \pm 0.24$  mg/g MLSS), and EPS is the main contaminant that causes membrane fouling. Therefore, EPS contributes more to membrane fouling than SMP in MBR systems because polysaccharides are considered to be the key substances responsible for membrane fouling, and proteins can contribute significantly to irreversible pollution [41,42]. In the two reactors with different filler additions, the concentrations of SMP in the mixture decreased slightly but EPS had significant decrease (13.42%–15.08%). The decrease in EPS is mainly from the decrease in protein, the feeding of fillers changed the ratio of protein and polysaccharides in EPS (Table S1). At day 12 of each stage, the protein concentration in EPS in the system decreased by 15.05% (floating filler in #1MBR), 15.93% (combination filler in #1MBR), 16.43% (floating filler in #2MBR), and 17.96% (combination filler in #2MBR) compared to without filler. The ratio of protein to polysaccharide (PN/PS) decreased to 2.49 (5.32% decrease), 2.53 (3.80% decrease), 2.87 (13.29% decrease), and 2.85 (13.90% decrease) in order. This finding indicated that the filler feeding reduces the protein concentration and effectively changes the composition of EPS and SMP in the sludge mixture. The floating sponge filler reduced the concentration of hydrophobic proteins in EPS through its own adsorption and degradation of microorganisms attached to the surface, which improved the agglomeration capacity of the sludge mixture and facilitated the control of membrane fouling [43]. The combination filler

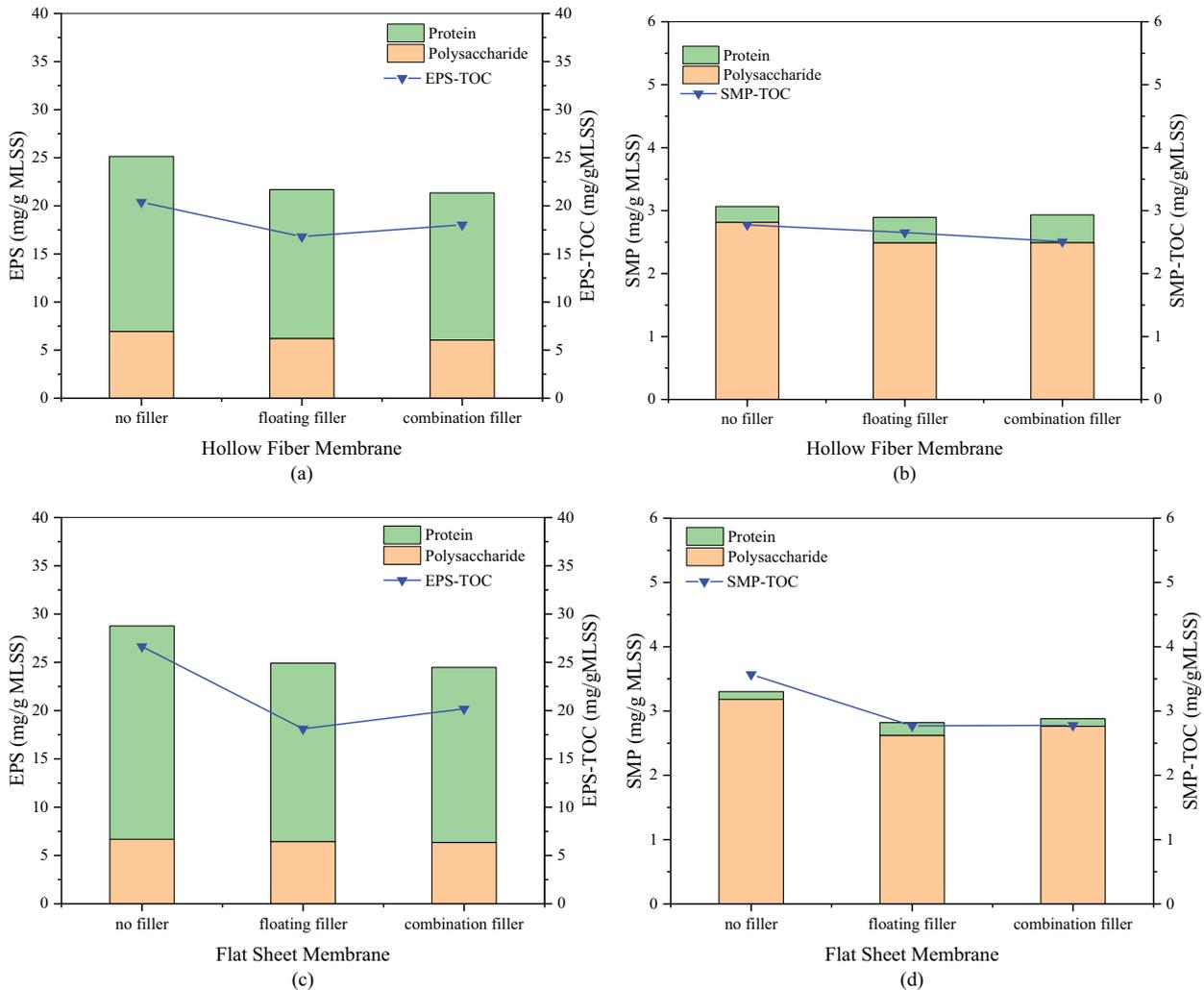


Fig. 4. Comparison of the EPS and SMP concentrations (mg/g MLSS) in #1MBR (hollow fiber membrane) and #2MBR (flat sheet membrane) with different fillers.

had similar ability, but the effect was not as obvious as the floating sponge filler probably because the combination filler itself lacked excellent adsorption ability and can only degrade the proteins and polysaccharides in the sludge mixture through the biofilm attached to it. The same conclusion can be obtained by observing the change in EPS in the MBR system after filling (Fig. S4). At the initial stage of floating sponge filling, the EPS concentration in the mixture decreased because of its strong adsorption capacity and then slightly increased because of the endogenous respiration of microorganisms [44]. The activated sludge was more stable, and the concentration of EPS in the mixture gradually decreased after the combination filler was fed.

### 3.4. Characterization of membrane fouling

Similar membrane resistance profiles and filter cake characteristics were observed in the two reactors (Table 2). The resistance of the filter cake layer accounts for 80% or more than 90% of the total resistance of the membrane, which is similar to previous studies [45]. The hollow fiber membrane

and flat sheet membrane systems can effectively mitigate membrane fouling by feeding floating sponge filler, and the effect is more significant in hollow fiber membrane systems. The floating sponge filler can adsorb some of the colloids and other biological macromolecules in the mixture due to its large specific surface area, and the air bubbles and filler can mechanically scour the membrane surface under the action of aeration, thereby inhibiting the formation of the cake layer and reducing its resistance (reversible resistance). However, the structure of the combination filler makes it easier for the sludge flocs to flocculate on it due to its large size, and the combination filler moves to the membrane surface and increases the membrane fouling with the action of hydraulic force.

FTIR can be used to analyze the functional groups of membrane surface cake layer and further clarify the membrane fouling [46]. The characteristic peaks of the FTIR spectra of hollow fiber membrane and flat sheet membrane filter cake layer were similar, and the main difference was the intensity of absorption peaks (Fig. 5). This finding is because the activated sludge and influent water of the

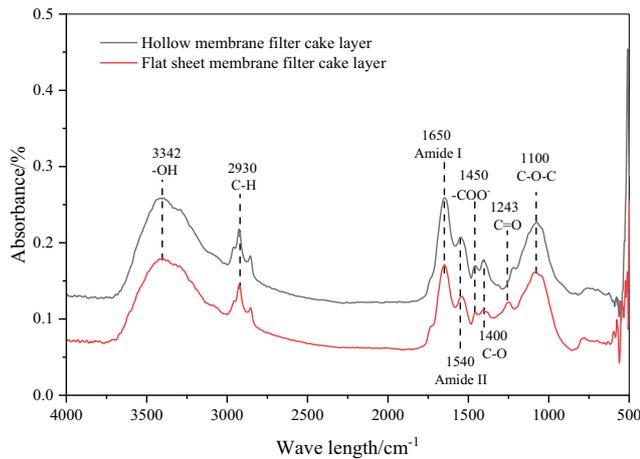


Fig. 5. Characteristic peaks of FTIR spectra of hollow fiber membrane and flat sheet membrane filter cake layer.

hollow and flat membrane bioreactor systems remain the same, showing that different types of membrane modules have the same retention effect on contaminants, and the membrane contaminants are mainly related to the influent water quality rather than the membrane module type. As shown in Fig. 5, eight main characteristic peaks were found in the FTIR spectra of the hollow fiber membrane and the flat sheet membrane filter cake layer. On the hollow fiber membrane filter cake layer, the main absorption peaks were 1,100; 1,243; 1,450; 1,650; 2,930 and 3,342  $\text{cm}^{-1}$ . The absorption peak at 1,100  $\text{cm}^{-1}$  was mainly related to the C–O–C bonds of polysaccharides [47], the absorption peak at 1,243  $\text{cm}^{-1}$  was mainly related to the C–N bonds in the carboxyl groups of proteins [48], the absorption peaks at 1,400 and 1,450  $\text{cm}^{-1}$  were mainly related to the stretching vibration of the  $-\text{COO}^-$  and C–O bonds of humic acid [47], the absorption peaks at 1,540 and 1,650  $\text{cm}^{-1}$  corresponded to the amide I and amide II bonds of the protein secondary structure, respectively [49], the absorption peak at 2,930  $\text{cm}^{-1}$  was mainly related to the stretching vibration of C–H [49], and the absorption peak at 3,342  $\text{cm}^{-1}$  was mainly generated by the stretching vibration of  $-\text{OH}$  [50]. Given that the characteristic peaks of the FTIR spectra of the filter cake layer of the flat sheet membrane and the FTIR spectra of the filter cake layer of the hollow fiber membrane were the same, the contaminants in the filter cake layer on the membrane surface of the hollow fiber membrane and the flat sheet membrane can be regarded as the same class of substances. In summary, the main pollutants in the membrane filter cake layer were protein, polysaccharide, and humic acid.

### 3.5. Analysis of microbial community

Feeding fillers into MBR can lead to improved nitrogen and phosphorus removal and can mitigate membrane fouling. Microbial sequencing was performed on the sludge mixture, floating filler and combination filler surface of hollow and flat membrane systems by using 16SRNA high throughput sequencing technology to further investigate the reason for this conclusion (Fig. 6). The dilution curves of microorganisms (Fig. S5) showed that the feeding of the

combination filler increased the diversity of microorganisms in the reactor, and that of the floating sponge filler was similar to that of the sludge mixture before performing the community analysis.

Fig. 6a describes the differences in the microbial community structure at the phylum level in the sludge mixture and on the fillers in two MBR systems. The dominant phyla were Proteobacteria, Actinobacteriota, Bacteroidota, Patensibacteria, Chloroflexi, and Acidobacteriota, and their total relative abundances in MBRH1 (sludge mixture in #1MBR), MBRH2 (floating filler in #1MBR), MBRH3 (combination filler in #1MBR), MBRF1 (sludge mixture in #2MBR), MBRF2 (floating filler in #2MBR), and MBRF3 (combination filler in #2MBR) were 93.55%, 91.81%, 93.09%, 91.22%, 93.44%, and 93.38%, respectively. This result is similar to previous studies on microbial community structure in MBR systems [51]. Proteobacteria accounted for 40.55% and 33.16% of the total relative abundance in MBRH1 and MBRF1, and was considered the main phyla for nitrification and denitrification [52]. The abundance of Proteobacteria on the combination filler was significantly higher than that in the sludge mixture (55.88% in MBRH3, 56.64% in MBRF3), indicating that its structure formed a more complete biofilm, which was conducive to the attachment and growth of nitrifying–denitrifying bacteria. By contrast, the abundance of Actinobacteriota was higher in the sludge mixture (38.48% in MBRH1, 37.97% in MBRF1) and the floating sponge filler (34.56% in MBRH2, 41.45% in MBRF2) than in the combination filler (17.38% in MBRH3, 19.31% in MBRF3). This finding may be because most of the Actinobacteriota are aerobic bacteria, and the thicker biofilm attached to the combination filler has an anoxic environment inside, which is un conducive to the growth of Actinobacteriota. Bacteroidota has the ability to degrade organic matter and possibly release the protein of EPS [53]. The MBR system fed with floating sponge filler had lower abundance of Bacteroidota (6.35% for MBRH2 and 4.17% for MBRF2) compared with MBRH3 and MBRF3 (8.56% and 8.63%), respectively, implying less EPS protein secretion and lighter membrane fouling (Fig. 4).

Fig. 6b shows the changes in community structure of sludge mixes and different filler sludge at the class level. In #1MBR and #2MBR, the dominant bacterial classes in the sludge mixture, floating sponge filler, and combination filler were Actinobacteria, Gammaproteobacteria, Alphaproteobacteria, Bacteroidia, and Saccharimonadia. In the #1MBR, the abundance of Saccharimonadia (6.55%) and Bacteroidia (5.57%) was higher in the floating sponge filler unlike the distribution of microbial communities in the sludge mixture. The abundance of Actinobacteria (14.84%) decreased, and the abundance of Gammaproteobacteria (38.3%), Alphaproteobacteria (17.48%), and Bacteroidia (8.35%) increased in the combination filler. In the #2MBR, the abundance of Saccharimonadia increased in the floating sponge filler, and the differences in the abundance of other classes were small. The abundance of Actinobacteria decreased more significantly, and Gammaproteobacteria, Bacteroidia, and Alphaproteobacteria increased more significantly in the combination filler. This finding is because Actinobacteria are aerobic bacteria [54], and the anoxic environment inside the combination filler is un conducive to growth. Gammaproteobacteria and Alphaproteobacteria are

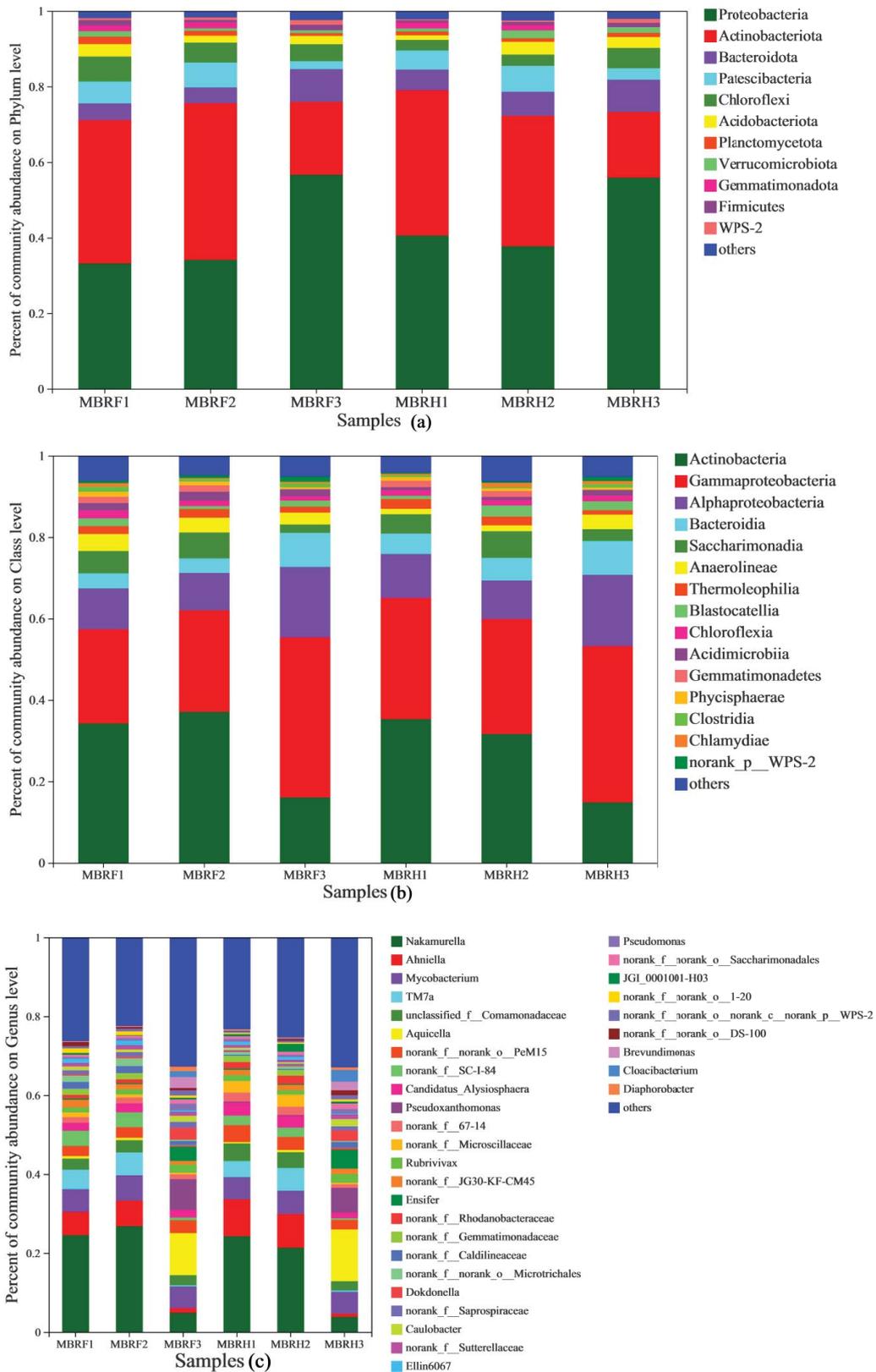


Fig. 6. Microbial community structure of MBRH1 (sludge mixture in #1MBR), MBRH2 (floating filler in #1MBR), MBRH3 (combination filler in #1MBR), MBRF1 (sludge mixture in #2MBR), MBRF2 (floating filler in #2MBR), MBRF3 (combination filler in #2MBR): (a) at phylum level, (b) at class level, and (c) at genus level.

a class of facultative anaerobe and aerobic bacteria, mainly decomposing organic matter, nitrogen, and phosphorus to provide energy for their own metabolism, with a strong denitrification capacity, which is conducive to denitrification [55]. Gammaproteobacteria and Bacteroidia were involved in secreting EPS and related to membrane fouling, which was validated by the permeation performance results (Fig. 3) [53,56].

Fig. 6c illustrates the variation of microbial diversity at the genus level. The structure of the microbial community on the floating sponge filler was similar to that of the sludge mixture. The dominant genus was *Nakamurella* (21.34%–26.80%), *Ahniella* (5.92%–9.32%), *Mycobacterium* (5.67%–6.42%), and *TM7a* (4.07%–5.79%). This finding indicates that this cubic structure of polyurethane floating sponge filler provides more attachment points for the activated sludge, but the powerful shear force caused by the intense aeration intensity hinders the formation of a stable and dense biofilm on the filler surface. A large amount of sludge mixture can be stored inside the filler due to the loose and porous structure of the sponge, which can affect the sequencing results. The abundance of species was mostly the same from that of the activated sludge, and this condition was similarly reflected in the Chao1 index (Fig. A4). The microbial community structure on the combination filler was relatively different from that of the sludge mixture and floating sponge filler. The abundance of *Nakamurella* (3.83% in MBRH3, 4.88% in MBRF3), *Ahniella* (0.83% in MBRH3, 1.15% in MBRF3), and *TM7a* (0.34% in MBRH3, 0.32% in MBRF3) showed a significant decrease. This finding may be due to the anoxic environment inside the combination filler that limits the growth of strictly aerobic bacteria, such as *Nakamurella* and *Ahniella* [57,58]. *TM7a* was involved in the interaction between carbon and nitrogen cycles [59], and its reduction was the reason why the nitrogen removal efficiency was not as excellent as expected. The abundance of *Aquicella* (13.10% in MBRH3, 10.63% in MBRF3) and *Pseudoxanthomonas* (6.26% in MBRH3, 7.78% in MBRF3) increased considerably. These microorganisms all have good denitrification ability [60] and maintain the nitrogen removal efficiency in the reactor at a high level.

#### 4. Conclusion

This study investigated the effects of different types of fillers (suspended and settled) fed into MBR in greywater treatment for water purification effect, membrane fouling, and microbial community structure. The conclusions are summarized as follows:

- Although the concentration of pollutants in the greywater are lower than domestic sewage, all of the MBR systems showed good purification. Among them, the MBR system with filler improved the removal rate of  $\text{NH}_3\text{-N}$ , TN, and TP compared with that without filler, especially the effect of floating sponge filler was obvious. Their average removal rates were 91.94%, 69.71%, and 66.41% in the hollow fiber membrane system, and were 91.49%, 71.53%, and 72.35% in the flat sheet membrane system. The effect of COD was mild, but its average effluent concentrations were maintained at low levels, which

were 29.35 mg/L in the hollow fiber membrane system and 27.53 mg/L in the flat sheet membrane system.

- The permeation performance of the MBR system with floating sponge filler was significantly improved and was conducive to the mitigation of membrane fouling. The feeding of combination filler was un conducive to the control of membrane fouling. When the hollow fiber membrane system was fed with floating sponge filler, the permeation performance at the end of each cycle ranged within  $1.0 \pm 0.2$  L/(m<sup>2</sup>·h·KPa) and returned to  $2.3 \pm 0.6$  L/(m<sup>2</sup>·h·KPa) at the beginning of next cycle. The permeation performance of the system with combination filler was similar to without filler and decreased rapidly during one cycle, ending with only  $0.25 \pm 0.1$  L/(m<sup>2</sup>·h·KPa).
- The concentration of EPS ( $25.06 \pm 3.71$  mg/g MLSS) was higher than that of SMP ( $3.06 \pm 0.24$  mg/g MLSS) in the sludge mixture. The main composition of EPS was protein ( $73.71\% \pm 3.10\%$ ), and polysaccharides were dominant in SMP ( $90.68\% \pm 5.70\%$ ). The filler feeding reduced the concentration of EPS in the sludge mixture and maintained good sludge volume characteristics in the MBR. The ratio of protein to polysaccharide (PN/PS) decreased to 2.49 (floating filler in #1MBR), 2.53 (combination filler in #1MBR), 2.87 (floating filler in #2MBR), and 2.85 (combination filler in #2MBR) at the end of each stage.
- The nutrients in the greywater can satisfy the normal growth of microorganisms, and at the same time, the addition of fillers changes their composition structure. A dense biofilm formed on the filler surface after feeding the combination filler (fixed type filler), and the anoxic environment inside led to a decrease in the abundance of some strictly aerobic bacteria, such as *Nakamurella* and *Ahniella* (3.83% and 0.83% in MBRH3, 4.88% and 1.15% in MBRF3). By contrast, the abundance of some denitrifying bacteria, such as *Pseudoxanthomonas* (6.26% in MBRH3, 7.78% in MBRF3) increased. In greywater treatment systems with low pollutant concentrations, the formation of biofilms on the surface of floating filler may take longer. The lower abundance of Bacteroidota (6.35% for MBRH2 and 4.17% for MBRF2) helps to reduce EPS secretion and mitigate membrane fouling.

#### CRedit authorship contribution statement

Chunhong Shi: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft, Funding acquisition. Cheng Wang: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft. Bo Yan: Investigation, Writing – Review & Editing, Project administration. Zifu Li: Conceptualization, Writing – Review & Editing, Supervision. Lixin Zhu: Resources, Project administration, Funding acquisition. Shuo Zhou: Conceptualization, Writing – Review & Editing. Xiaochen Wang: Conceptualization, Writing – Review & Editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supporting information



Fig. S1. Different fillers fed into the MBR system: (a) floating sponge filler and (b) combination filler.



Fig. S2. Schematic diagram of the microbial sequencing process.

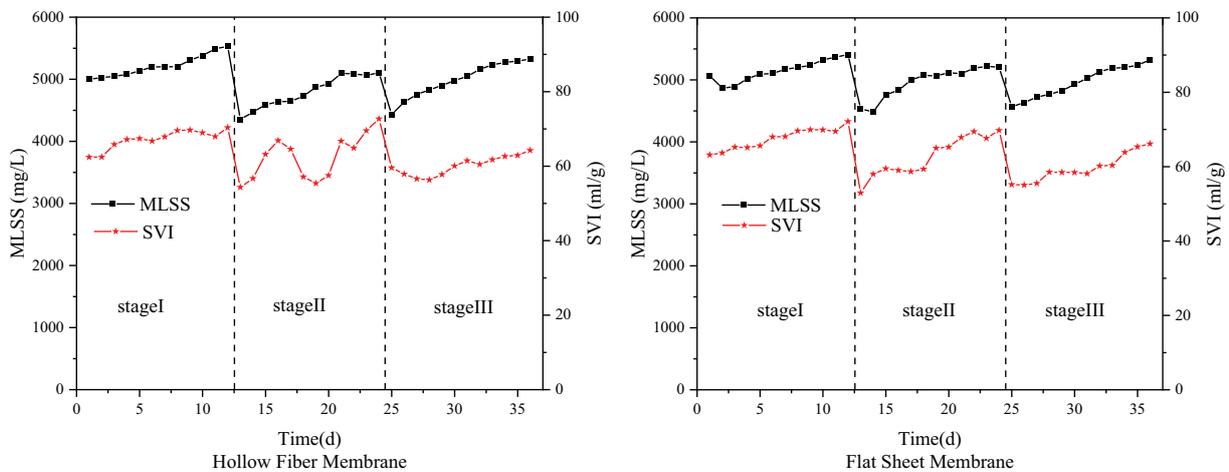


Fig. S3. Change of the sludge concentration of the hollow fiber membrane and the flat sheet membrane after the feeding of different fillers: (stage I) no filler, (stage II) floating sponge filler, (stage III) combination filler.

Table S1  
Change of the ratio of protein and polysaccharides in EPS through feeding different fillers

Filler	EPS					
	Hollow fiber membrane #1MBR			Flat sheet membrane #2MBR		
	PN (mg/g MLSS)	PS (mg/g MLSS)	PN/PS	PN (mg/g MLSS)	PS (mg/g MLSS)	PN/PS
No filler	18.21	6.93	2.63	22.10	6.67	3.31
Floating filler	15.47	6.21	2.49	18.47	6.44	2.87
Combination filler	15.31	6.04	2.53	18.13	6.35	2.85

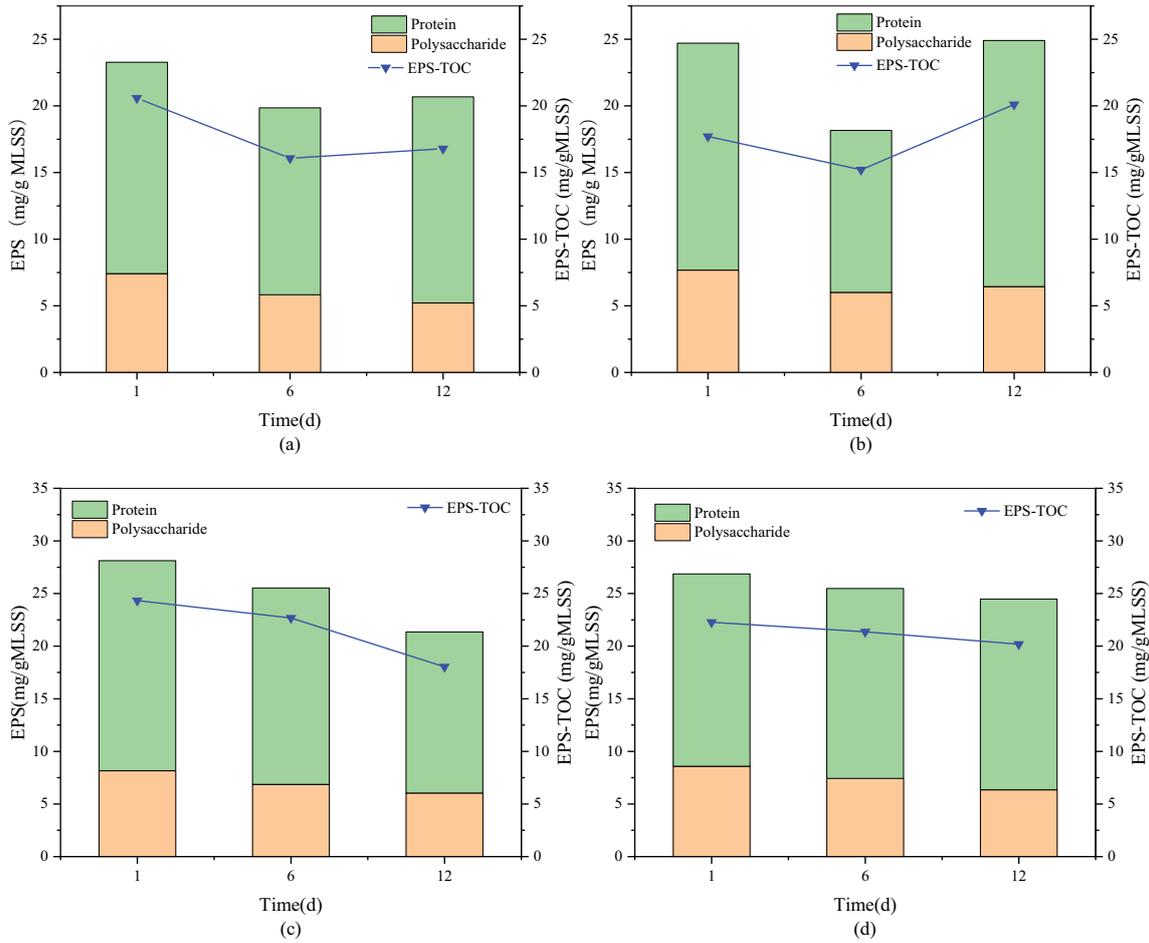


Fig. S4. Change of EPS in the MBR system after filling: (a) #1MBR fed with floating sponge filler, (b) #2MBR fed with floating sponge filler, (c) #1MBR fed with combination filler, and (d) #2MBR fed with combination filler.

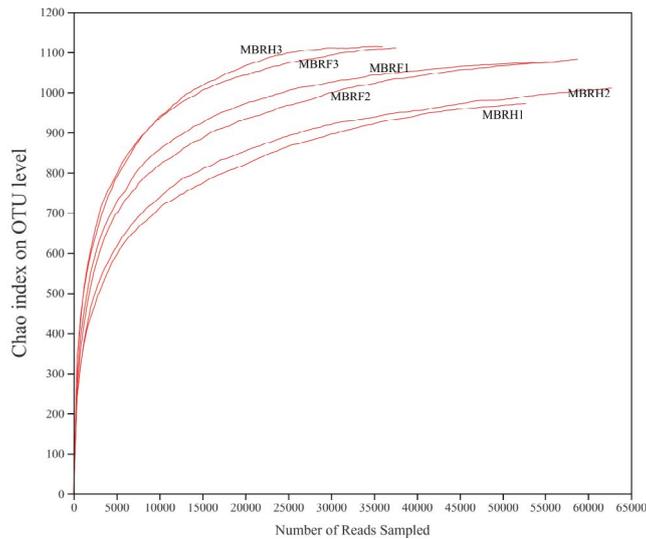


Fig. S5. Dilution curves of microorganisms: MBRH1 (sludge mixture in #1MBR), MBRH2 (floating filler in #1MBR), MBRH3 (combination filler in #1MBR), MBRF1 (sludge mixture in #2MBR), MBRF2 (floating filler in #2MBR), MBRF3 (combination filler in #2MBR).