

# Toward Self-sustaining Membrane Filtration via Manipulating the Membrane-surface Ecosystem

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**Abstract:** Biofouling-induced membrane contamination severely limits the application of membrane filtration systems. Regulating biofilm formation on membrane surfaces can facilitate the development of water channels and help establish a dynamic balance between foulant deposition and biodegradation. In this study, an activated carbon fiber (ACF) barrier and a biofiltration-integrated ACF barrier (BF\_ACF) were employed to evaluate their effects on the performance of a gravity-driven membrane (GDM) ultrafiltration (UF) system. The results showed that ACF and BF\_ACF, significantly improved dissolved organic matter (DOM) removal, increasing removal efficiencies from approximately 5–13% (for UF alone) to 21–25% and 78–81%, respectively. In addition, the steady-state membrane fluxes of the ACF and BF\_ACF systems increased substantially from  $1.96 \pm 0.15$  (UF alone) to  $2.84 \pm 0.22$  and  $4.19 \pm 0.13$  LMH, respectively, corresponding to increases of approximately 44.9% and 214% relative to the control. ACF and BF\_ACF were found to alter the heterogeneity of the biofilm layer, resulting in a thinner, looser, and more porous structure. A shift in the microbial community from r-selected to K-selected taxa was also observed, transforming the membrane-surface ecosystem from a “fouling-type” community into a “cleaning-type” community. Overall, this study provides a paradigm for “controlling fouling with microorganisms” through the artificial regulation of the membrane-surface ecological niche, thereby improving the sustainability of membrane-based water treatment systems.

**Keyword:** Water treatment; Membrane filtration; Dissolved organic matter; Activated carbon fiber; Biofilm; Microbial diversity

## 30 **1. Introduction**

31 Access to safe drinking water remains a major global challenge, particularly in low-  
32 income countries and rural areas lacking centralized water supply systems (Mu et al., 2023).  
33 Gravity-driven membrane (GDM) systems offer several advantages, including ultra-low  
34 energy consumption, simple operation, high reliability, and cost-effectiveness, making them an  
35 attractive and environmentally friendly option for decentralized water supply.

36 The performance of GDM systems is largely determined by the biofilm that forms on the  
37 membrane surface (Li et al., 2022). During membrane filtration, the introduction of organic  
38 matter and bacteria inevitably leads to the development of a biofilm layer (Tang et al., 2018).  
39 This layer provides a favorable environment for microbial enrichment and creates water  
40 channels, while also enabling the biodegradation of organic contaminants (Kim et al., 2025).  
41 However, the limited hydraulic retention time restricts contaminant removal efficiency. In  
42 addition, an unregulated biofilm layer can increase hydraulic resistance and impede continuous  
43 high-throughput operation, thereby compromising the overall sustainability of GDM processes  
44 (Desmond et al., 2018).

45 In situ and external regulation are the two main strategies used to improve the performance  
46 of membrane filtration processes. In situ control involves various techniques, including  
47 aeration, electrochemical oxidation, and pre-deposition (Braak et al., 2011; Fang et al., 2025;  
48 Song et al., 2024), which generally function by altering contaminant properties and modifying  
49 the interactions between the biofouling layer and the membrane (Tominaga et al., 2022).  
50 Similar to pre-deposition (Nava-Ocampo et al., 2020), a pre-arranged adsorptive functional  
51 barrier on the GDM membrane surface offers multiple advantages, such as the pre-removal of  
52 larger impurities through adsorption and interception, as well as the provision of a medium for  
53 biodegradation. Moreover, this strategy requires no additional operational steps, making it a  
54 promising approach for enhancing GDM system performance.

55 Various adsorbents, such as powdered activated carbon (PAC), carbon nanotubes, and  
56 porous resins, have been employed to mitigate membrane fouling (Anantharaman et al., 2020).

These adsorbents share several common features, including porous structures, strong adsorption capacities, and the ability to support microbial colonization (Cai et al., 2013; Li et al., 2020). Activated carbon fiber (ACF), an advanced derivative of conventional activated carbon, possesses abundant micropores, an excellent volumetric adsorption capacity, and a substantially larger specific surface area (Joo et al., 2025; Shin et al., 2026; Yaqub and Lee, 2020). Compared with PAC, the micropores of ACF are predominantly distributed on the fiber surface (Prajapati et al., 2016), resulting in faster adsorption rates, shorter adsorption pathways, and lower mass-transfer resistance. Therefore, ACF is more suitable for applications with short hydraulic retention times, such as membrane filtration processes. In addition, the fibrous network structure of ACF allows it to be readily integrated onto the membrane surface, making it a suitable material for constructing an adsorptive functional barrier on membrane surfaces.

External regulation methods, including coagulation, ozonation, and biofiltration, have also been widely used as a pretreatment to improve membrane filtration performance (Feng et al., 2022; Jin et al., 2019b). Among these approaches, biofiltration (e.g. biological activated carbon, BAC) is an economical and environmentally friendly process that requires no chemical addition (Nabaterega et al., 2025), and can be readily integrated with GDM systems, particularly for decentralized applications in rural areas. Biofiltration is known to contribute to the pre-purification of raw water by removing natural organic matter and other contaminants, which may directly affect the biofouling layer formed on the membrane surface during long-term operation (Pronk et al., 2019). However, how biofiltration influences biofilm development in GDM systems equipped with an ACF barrier, and how these changes affect overall GDM performance, remains unclear.

In this study, we first investigated the effect of using ACF as a barrier on the GDM membrane surface and evaluated its impact on system performance. To further enhance system performance, viability and sustainability, we subsequently considered the application of biofiltration as a pretreatment for the ACF-GDM system (BF\_ACF). The resulting effects on membrane-surface biofilm formation, membrane flux, and effluent water quality were systematically examined. Microbial diversity and metabolic pathways were further analyzed to

elucidate the underlying relationships among water quality, biofilm structure, and microbial communities. This work provides not only a feasible paradigm for decentralized GDM systems in rural areas, but also insights into a broader ecological principle: how water quality shapes microbial diversity and, consequently, biofilm structure. These findings may inform a wide range of engineering practices.

## **2. Materials and Methods**

### **2.1. Feeding water**

Natural raw water was collected weekly from a local lake (Beijing Olympic Park, China, main characteristics were provided in Supplementary Information (SI), Table S1). The water samples were transported to the laboratory and analyzed immediately. Prior to storage at 4 °C, the samples were filtered through lint-free cotton gauze to remove visible impurities and particles. During operation, the feed water was first equilibrated to room temperature (~22 °C) and then directly supplied to the GDM systems. However, for the biofiltration-integrated GDM system (BF-ACF), the same raw water was used as the influent to a separate, previously established BAC pretreatment stage and the effluent from this biofiltration process was collected and applied as influent to the ACF-GDM system; details of the BAC pretreatment have been reported previously (Chen et al., 2025), and provided in SI, Text S1.

### **2.2. System setup and operation**

The GDM system was custom-designed, with detailed configuration illustrated in the SI (Figure S1). Three systems were operated continuously in parallel for 62 days for comparison; these were: UF, representing the conventional GDM system; ACF, in which ACF was placed as a barrier on the membrane surface; and BF\_ACF, in which the ACF-equipped UF system was fed with biofilter effluent (BF). Specifically, a membrane with an effective filtration area of 153.94 cm<sup>2</sup> (14 cm in diameter) was placed on a perforated support, followed by the ACF barrier (in the ACF and BF\_ACF systems) and a silicon rubber gasket. The assembly was then sealed with bolts. The UF membrane used in this study was made of polyvinylidene fluoride (PVDF) with a molecular weight cut-off of 100 kDa. ACF was purchased from Beierge

Activated Carbon Fiber Co., Ltd. (Nantong, China), and its basic properties are listed in the SI, Table S2. Prior to use, the UF membranes were soaked in ultrapure water to remove impurities. Permeate from the GDM systems was collected in glass bottles for subsequent analyses, including membrane flux measurement and water quality assessment.

### **2.3. Water quality analysis**

Water samples were collected and filtered through 0.45 µm syringe filters before analysis. Dissolved organic carbon (DOC) was measured using a total organic carbon analyzer (TOC-Vwp, Shimadzu, Japan). The fluorescence intensity of fluorescent organic matter was characterized using three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy (F-4600 FL, HITACHI, Japan). Specific fluorescent components were quantified using parallel factor analysis (PARAFAC) and fluorescence region integration. In addition, the apparent molecular weight (MW) distribution of dissolved organic matter (DOM) was determined using a high-performance liquid chromatography (HPLC) system (Waters, USA) coupled with a size-exclusion chromatography (SEC) column (Phenomenex, BioSep 5 µm, SEC-s3000). Details of the measurements are provided in the SI (Text S2).

For determining the reduction in disinfection byproduct formation potential (DBPFP) of treated waters, samples of the raw water and GDM effluents were collected and chlorinated separately. The DBPFP was determined based on commonly detected trihalomethanes (THMs) and haloacetic acids (HAAs), in order to assess DOM transformation and the ability of the systems to remove DBP precursors. Detailed procedures are provided in the SI (Text S3).

### **2.4. Membrane filtration performance**

#### **2.4.1. Flux**

The permeation flux of the ultrafiltration system,  $J$  [L/(m<sup>2</sup>·h), LMH], was calculated using the following equation:

$$J = \frac{\Delta V}{A \cdot \Delta T}$$

where  $\Delta V$  (L) and  $A$  (m<sup>2</sup>) represent the volume of filtered liquid collected during the period of operation  $\Delta T$  (h), and the effective filtration area, respectively.

#### **2.4.2. Hydraulic resistance**

The total hydraulic resistance,  $R_t$  ( $\text{m}^{-1}$ ), was determined as follows:

$$R_t = \frac{TMP}{\mu \cdot J}$$

where  $TMP$  is the transmembrane pressure, corresponding to a 65-cm water head, and  $\mu$  ( $\text{Pa} \cdot \text{s}$ ) is the dynamic viscosity of water at the corresponding temperature.

## **2.5. Biofilm Characterization**

### **2.5.1. Scanning electron microscopy and X-ray photoelectron spectroscopy**

At the end of the experiment, the fouled UF membranes were removed and cut into several pieces for different analyses. One piece was freeze-dried and carefully cut into small segments. A platinum coating was applied to enhance the electrical conductivity of the membrane samples. Surface and cross-sectional morphologies of the fouled membranes were observed using high-resolution field-emission scanning electron microscopy (FE-SEM, SU 8000, HITACHI, Japan). The thickness of the cross-sectioned membrane biofilm was measured in triplicate using DigitalMicrograph software (GATAN AMETEK, USA). For chemical bonding analysis, naturally dried membranes containing the biofilm layer were analyzed using X-ray photoelectron spectroscopy (XPS, Thermo Scientific 250Xi).

### **2.5.2. Extracellular polymeric substances extraction**

Extracellular polymeric substances (EPS) in the biofouling layer were extracted using a heating method (Xiong et al., 2021). Briefly, the membrane was first rinsed with phosphate-buffered saline (PBS), then suspended in 20 mL of PBS solution and vortexed for 10 min. After vortexing, the rinsed membrane samples were removed. The remaining suspension was heated at 80 °C for 30 min and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected for subsequent analysis.

### **2.5.3. Microbial community analysis**

At the end of the experiment, biofilm samples were collected and stored in sterile centrifuge tubes at -20 °C for DNA extraction and 16S rRNA gene sequencing. After high-throughput sequencing, the obtained data were filtered, and all samples were normalized to 18,402 sequences. Microbial community structure and diversity were processed and analyzed using the Majorbio Cloud Platform. Principal coordinate analysis (PCoA), and the Kruskal–

Wallis H test were performed to assess significant differences among multiple groups. PICRUST2 was used to predict the potential functions of the bacterial communities based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The raw sequencing data were deposited in the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) under accession number PRJNA1395702.

### **3. Results and Discussion**

#### **3.1. DOM Removal by GDM systems operated under different regimes**

DOM is a critical water quality parameter affecting the performance of GDM systems and therefore requires careful consideration. To evaluate variations in influent and effluent water quality and removal efficiency, influent and effluent samples were collected and analysed after approximately two weeks of operation, when the flux and biofouling layer had become relatively stable. Figure 1a shows the DOM removal efficiencies of the different GDM systems and individual processes. It can be observed that the influent and effluent DOM characteristics of each system remained generally stable throughout the operation period, with only minor fluctuations. Specifically, the UF system removed approximately 5–13% of DOM from raw water, mainly through the interception of high-molecular-weight organic matter, such as proteins, polysaccharides and colloidal organics (Henderson et al., 2011). The ACF system showed improved DOM removal, reaching approximately 21–25%, which could be ascribed to both adsorption and biodegradation (Ma et al., 2026). In the BF\_ACF system, the DOM removal rate increased significantly to around 80%. Compared with the effluent from the BF alone, the BF\_ACF effluent showed that an additional 5–8% DOM removal was achieved by the subsequent ACF-GDM. These results indicate that the ACF barrier is a simple but effective strategy for enhancing DOM removal in GDM systems, while BF can serve as a robust pretreatment step when integrated with GDM systems for DOM removal.

Further analysis of the molecular weight (MW) distribution of DOM showed that UF had limited organic matter removal, whereas ACF effectively removed small-MW organic compounds, particularly those with MW around 1 kDa. In contrast, BF pretreatment exhibited

a robust and highly efficient organic matter removal across the entire MW range. The subsequent ACF-GDM in the BF\_ACF system achieved further removal of small-MW organic compounds. This sustained removal was mainly attributed to the synergistic effects of adsorption by ACF and subsequent biodegradation by microorganisms. ACF selectively adsorbed organic matter, while the attached microorganisms degraded the adsorbed compounds, forming an effective adsorption–biodegradation cycle (Ma et al., 2026). Moreover, the preferential removal of small-MW organic compounds by ACF and BF\_ACF likely resulted from the abundant microporous structure of ACF, which provided a selective adsorption capacity.

### Figure 1.

Analysis of fluorescence intensity in the effluent samples further indicated that ACF had a certain degree of effectiveness in removing fluorescent organic matter. Specifically, PARAFAC analysis identified four fluorescent components (Fig. 1c). Among them, C1 exhibited excitation and emission wavelengths characteristic of microbial humic-like substances in DOM, while C2 and C3 were associated with protein-like and humic-like components, respectively. C4 was associated with tyrosine-like and/or tryptophan-like substances and may also reflect microbial byproduct-like organic matter, with excitation and emission similarity scores exceeding 0.95 (Retelletti Brogi et al., 2019; Sharma et al., 2017; Shutova et al., 2014; Wünsch et al., 2017). The UF system alone showed limited removal of all components, whereas BF efficiently removed all components, especially C1 and C3. In contrast, ACF primarily targeted the removal of C2 and C4. Therefore, even after robust BF pretreatment, the ACF barrier still played a critical role in reducing low-molecular-weight organic matter and/or soluble microbial products, contributing to a better-performing and more sustainable GDM system.

### 3.2. Membrane flux and effluent DBPFP

The changes in UF membrane flux and total hydraulic resistance were investigated to evaluate membrane fouling development under different scenarios (Fig. 2a and Fig. S2). In

constant-pressure dead-end filtration, membrane fouling is typically caused by the simultaneous occurrence of pore blockage, pore narrowing, and cake layer formation, with the dominant mechanism shifting over time during operation (Yu et al., 2018). The results showed that flux variation in the GDM systems generally progressed through three phases: rapid decline (0–6 days), pseudo-steady state (6–17 days), and flux stabilization (17–62 days) (Lin et al., 2022). Compared with the UF system, which had a stable flux of  $1.96 \pm 0.15$  LMH, the ACF system increased the stabilized flux by approximately 44.9%, reaching  $2.84 \pm 0.22$  LMH. For the BF\_ACF system, the initial flux was relatively higher, and the flux remained higher than those of the other two systems throughout the entire operation period. The final stable flux was approximately 2.14 times that of the UF system, reaching  $4.19 \pm 0.13$  LMH. The trend in total hydraulic resistance was consistent with the flux results (Fig. S2), with all GDM systems initially exhibiting a rapid increase in resistance followed by gradual growth. During the steady-state period, the total hydraulic resistance of the UF system was approximately 1.5 and 2.65 times those of the ACF and BF\_ACF systems, respectively. These results further demonstrate that BF pretreatment combined with an ACF barrier could be an effective strategy for improving the feasibility and sustainability of GDM systems.

## Figure 2.

The formation of disinfection byproducts is an important factor in evaluating the effluent suitability for different uses (Fig. 2b, c). For the THMFP, its reduction by the UF system was mainly driven by the decrease of DOM associated with biofilm formation (Wu et al., 2024). Compared with the UF system, the ACF system exhibited an improved performance, which was related to interception and microbial degradation within the ACF barrier. Previous studies have shown that humic substances with MW values ranging from 3 to 5 kDa contain abundant polyhydroxy aromatic structures and exhibit high reactivity toward chlorine (Nguyen et al., 2021). Consequently, the reduction of the C3 component by the ACF barrier contributed to a decrease in the THMFP. For the BF\_ACF system, the BF pretreatment removed a substantial amount of DOM, which in turn greatly reduced the THMFP. The subsequent ACF further

removed low-MW organic compounds, achieving an approximately 82.5% reduction in THMFP overall for the BF\_ACF system compared with the raw water, from  $526.60 \pm 33.18$  to  $92.19 \pm 8.89$   $\mu\text{g/L}$ . For HAAFP, biopolymers were largely intercepted on the UF membrane surface (Tominaga et al., 2022), whereas UF showed limited removal of low-MW organic matter. Previous studies have shown that low-MW organic compounds are major precursors of HAAs (Zhang et al., 2014). BF and ACF demonstrated strong abilities to remove low-MW organic compounds, including aldehydes and ketones that can readily react with chlorine to form HAAs (Bond et al., 2011). As a result, BF\_ACF achieved an extremely high HAAFP removal of approximately 95.3%, corresponding to a decrease from  $348.74 \pm 38.18$  for the raw water to  $16.27 \pm 3.33$   $\mu\text{g/L}$ .

Linear regression and Mantel tests (Fig. 2d) showed that DOM concentration was a key factor influencing flux performance ( $R^2 = 0.825$ ), with the C2 and C4 components being more decisive contributors ( $p < 0.05$ ). Previous studies have also shown that, under identical conditions, higher DOM concentrations result in greater retention on the membrane surface, thereby affecting biofilm thickness and reducing permeation flux (Lin et al., 2009). Regarding DBPFP, the DOM and  $\text{UV}_{254}$  were key factors influencing the DBPFP and exhibited strong positive correlations, particularly with aromatic components ( $p < 0.01$ ). Components C1 and C3, representing humic-like substances, were the dominant fluorescent fractions influencing the formation of both THMFP and HAAFP ( $p < 0.05$ ). This is consistent with previous studies reporting a strong linkage between humic substances and DBPFP formation (Hao et al., 2012).

### 3.3 Morphology and characterizations of biofilms formed in different GDM systems

The surface and cross-sectional morphologies of the biofilms formed in each system were observed by SEM at the end of the experiment. Compared to the other systems, the UF system biofilm exhibited a thicker and denser fouling layer, characterized by large amounts of EPS-embedded microorganisms, and with an average thickness of approximately  $300.38 \pm 9.36$   $\mu\text{m}$ . For the ACF and BF\_ACF systems, the fouling layer was less sticky and more distinct and flatter, with the thickness decreasing substantially to approximately  $188.73 \pm 4.73$   $\mu\text{m}$  and  $86.52 \pm 3.94$   $\mu\text{m}$ , respectively (Figure 3). Moreover, the cross-sectional profiles showed that

the biofilm formed in the BF\_ACF system was significantly looser and more porous than those in the UF and ACF systems, indicating improved structural heterogeneity.

It is hypothesized that, on the one hand, the ACF barrier provides a suitable growth environment for microorganisms, thereby promoting organic matter biodegradation and a reduction in the thickness of the biofouling layer (Feng et al., 2022). On the other hand, the ACF barrier prevents direct interaction between the feed water and the UF membrane, which may reduce the hydraulic pressure exerted on the biofilm and consequently decrease its compactness and thickness (Shao et al., 2017). In the BF\_ACF system, due to the efficient pre-removal of DOM by the biofilter, the amount of residual biodegradable organic matter available for microbial growth on the membrane surface was greatly limited. To achieve more efficient mass transfer within the biofilm, including the transport of nutrients and oxygen, the biofilm structure may therefore become more open. In addition, reduced EPS accumulation can weaken the bridging and adhesion effects among colloids, leading to a more porous structure. Furthermore, nutrient limitation may enhance endogenous respiration, thereby triggering internal biodegradation and restructuring of the biofilm and making it more porous.

### Figure 3.

As the initial attachment of biopolymers and/or soluble microbial products (SMP) to the membrane surface is an important factor influencing subsequent biofilm formation (Xu et al., 2021), the accumulation of biopolymers on the membrane surface was further examined (Fig. 3j). The results clearly showed that the UF system exhibited the highest polysaccharide and protein contents, reaching  $40.73 (\pm 1.74) \times 10^2$  and  $8.46 (\pm 0.43) \times 10^2$  mg/m<sup>2</sup>, respectively. For the BF\_ACF system, the polysaccharide and protein contents were greatly reduced by more than 98% and 96%, respectively. Fluorescence analysis revealed similar peaks but different intensities among the systems, and SMP were identified as the major components of the biofilm layer (Fig. 3k and Fig. S3). Higher SMP levels were associated with relatively higher EPS accumulation, which in turn exacerbated the reduction in biofilm porosity (Desmond et al., 2022).

The hydrophilicity of the fouling layer was also examined to understand its influence on biofilm formation (Fig. 31). The results indicated that substantial hydrophobic substances were deposited on the UF membrane after long-term operation, as reflected by an increase in the contact angle to  $112.04 \pm 3.86^\circ$ , compared with approximately  $80^\circ$  for the pristine UF membrane. This may be attributed to the gradual displacement of initially adsorbed hydrophilic microbial byproducts, such as polysaccharides, by hydrophobic components such as humic acids during prolonged operation, or to the embedding of hydrophobic substances within the pores of the hydrophilic deposit layer (Zhang et al., 2022). In contrast, the contact angles of the ACF and BF\_ACF systems decreased significantly to  $67.04 \pm 2.91^\circ$  and  $44.23 \pm 1.49^\circ$ , respectively. This suggests that both the BF process and the ACF barrier effectively removed hydrophobic organic matter from the raw water through biodegradation, physical interception, and adsorption (Huang et al., 2024; Zhang et al., 2020; Zularisam et al., 2007).

XPS was also employed to analyze the biofilm layer on the membrane surface and obtain detailed information with respect to chemical bonding (Fig. 4). In the C 1s spectra, the C1 peak was assigned to C–C/C–H bonds, mainly originating from hydrocarbon compounds, including the side chains of polysaccharides, amino acids, and lipids (Zhang et al., 2024). The C2 peak corresponded to C–O/C–N bonds in amide, alcohol, or ether groups within proteins and polysaccharides (Wang et al., 2022). The weak C3 peak was attributed to RCOOH/O=C–O–R groups, such as carboxyl or ester groups in carboxylates and sugars (Xu et al., 2023).

#### Figure 4.

Compared with the UF system, the C1 component in the ACF system decreased from 48.60% to 36.27%, likely due to the microbial degradation of macromolecules into amides, alcohols, and other substances. Meanwhile, the C2 component increased from 33.78% to 57.99%. The C3 component decreased from 17.62% to 5.74%, indicating the removal of carboxylates within the ACF barrier, possibly through biodegradation or adsorption. For the BF\_ACF system, the C1 component remained low at 36.65%, while the proportion of C3 increased significantly to 25.80%. This could be attributed to greater biodegradation during BF

pretreatment, which converted more C–O/C–N organic compounds into O–C=O/O=C–N compounds (Tansel et al., 2006; Uning et al., 2025). This finding is also consistent with the changes in membrane contact angle, which showed increased hydrophilicity.

For the O 1s spectra, the peaks were assigned to C=O and C–O–H/C–O–C at 531.2 and 532.4 eV, respectively. These groups represent proteins, peptides, carboxylates, humic substances, polysaccharides, alcohols, and ethers. Compared with the UF system, the proportion of C=O species increased in the ACF and BF\_ACF systems from 24.35% to 42.50% and 30.66%, respectively, while the proportion of C–O–H/C–O–C species decreased from 75.65% to 57.50% and 69.34%, respectively. This could primarily be attributed to the microbial transformation of C–O components into C=O species (Li et al., 2022). However, the C=O component in the BF\_ACF system was slightly lower than that in the ACF system, accompanied by a corresponding increase in C–O–C/C–O–H components. This may have resulted from the significant removal of humic substances while maintaining a comparable SMP content (Fig. 1d) (Okamura et al., 2010).

### 3.4 Microbial diversity and similarity analysis

Microbiome analysis was conducted to elucidate the effects of the ACF barrier and BF pretreatment on microbial diversity within the biofilm formed on the UF membrane surface at the end of operation. Differences in microbial diversity among the systems are shown in Fig. 5. The results showed that 65 ASVs were shared by all three systems, accounting for only 51.2% of the total ASVs in the UF system, 52.0% in the ACF system, and 49.2% in the BF\_ACF system, with total ASV numbers of 127, 125, and 132, respectively. In addition, the BF\_ACF system exhibited the most distinct microbial diversity compared with the UF and ACF systems, with approximately 40% unique ASVs. These results indicate that both the ACF barrier and BF pretreatment altered the microbial diversity of the biofilm formed on the UF membrane surface, with BF pretreatment exerting a stronger influence.

### Figure 5.

Principal coordinate analysis (PCoA) based on Bray–Curtis distance indicated significant

differences in microbial community composition among the systems (ANOSIM,  $R = 1$ ,  $P = 0.003$ ) (Fig. 5b).  $\alpha$ -Diversity analysis, including the Chao and Simpson indices, further revealed that the ACF barrier and BF pretreatment reduced species richness but increased microbial diversity within the biofilm layer, with all coverage indices exceeding 0.99 (Fig. 5c, d and Fig. S4). This phenomenon is consistent with the enrichment of dominant species in the BF system observed in our previous study (Huang et al., 2024). Community similarity analyses based on three distance matrices, i.e. Bray-Curtis (Fig. 5e), weighted and unweighted UniFrac (Fig. 5f), showed that environmental factors exerted a certain regulatory effect on the abundance distribution of dominant taxa (Bray-Curtis,  $R^2 = 0.356$ ). The dominant taxa exhibited similar phylogenetic relationships, indicating that highly abundant species were phylogenetically close to one another (weighted UniFrac,  $R^2 = 0.176$ ). However, the phylogenetic structure of rare taxa represented the primary feature driving community differentiation, with differences among rare taxa being much greater than those among dominant taxa. A large number of unique evolutionary branches were present among different sample groups, and these branches were mainly derived from rare species (unweighted UniFrac,  $R^2 = 0.889$ ).

### 3.5 Dominant microbial species within the biofilms

Microbial diversity was further analyzed at different taxonomic levels (Fig. 6, Figs. S5–S7). The results of the Kruskal–Wallis H test showed that both the ACF and BF\_ACF barriers significantly altered microbial community composition at the phylum level ( $p < 0.05$ ) (Fig. S8). Specifically, the phyla *Bacillota* and *Gemmatimonadota* were significantly enriched in the BF\_ACF system compared with the UF and ACF systems, increasing from less than 0.05% to  $8.71 \pm 5.04\%$  and  $2.07 \pm 0.23\%$ , respectively (Fig. 6a). In addition, *Actinomycetota* and *Acidobacteriota* were also enriched in the BF\_ACF system, with relative abundances of  $8.73 \pm 3.51\%$  and  $3.48 \pm 1.72\%$ , respectively, compared with less than 2.5% and 0.2% in the ACF system. Previous studies have shown that *Bacillota* and *Gemmatimonadota* have strong capacities for decomposing complex carbon compounds and polysaccharides in natural environments (López-Mondéjar et al., 2016; Mujakic et al., 2023), while *Actinomycetota* and

*Acidobacteriota* can secrete a range of extracellular enzymes and metabolize recalcitrant organic matter (Mccarthy and Williams, 1992; Reji and Zhang, 2022). Therefore, the microbial community appeared to shift toward a more efficient biodegradation regime, which could directly promote DOM removal and fouling mitigation during filtration. This trend was further supported by ternary analysis at the order level, which showed that *Gemmatimonadales*, belonging to *Gemmatimonadota*; *Bacillales* and *Clostridiales*, belonging to *Bacillota*; and *Mycobacteriales*, belonging to *Actinomycetota*, were the dominant taxa in the BF\_ACF system (Fig. 6b).

At the genus level, it is noteworthy that the top 4 genera in the UF system, namely *g\_norank\_f\_Caldilineaceae*, *g\_norank\_f\_SM2D12*, *g\_norank\_f\_Micropepsaceae*, and *g\_Polynucleobacter*, and the top 5 genera in the BF\_ACF system, namely *g\_Methyлотenera*, *g\_Hydrogenophaga*, *g\_Mycobacterium*, *g\_Methylophilus*, and *g\_Rhodofera*, accounted for more than 55% of the total microbial abundance in their respective systems ( $55.92 \pm 3.16\%$  and  $56.41 \pm 3.48\%$ , respectively). This indicates a substantial transformation of the microbial community structure.

In the UF system, the microbial community was dominated by taxa associated with nutrient utilization and active basic metabolism. For example, *g\_norank\_f\_Caldilineaceae* belongs to filamentous bacteria that can contribute to the formation of dense biofilms (Sekiguchi and Hanada, 2015), *g\_norank\_f\_SM2D12* is often considered an energy-parasitic taxon and may enhance carbon fixation within the biofilm (Schön et al., 2022), and *g\_norank\_f\_Micropepsaceae* has strong glycolytic capability (Harbison et al., 2017), while *g\_Polynucleobacter* is widely distributed in freshwater systems and mainly utilizes humic substances for growth (Hahn et al., 2012). In contrast, the BF\_ACF system was characterized by efficient biodegraders; specifically, *g\_Hydrogenophaga*, *g\_Mycobacterium*, and *g\_Rhodofera* are known to be effective degraders of aromatic compounds and play key roles in the initial degradation and ring cleavage of aromatic pollutants (Banerjee et al., 2021; Hennessee and Li, 2016; Tancsics et al., 2023). Meanwhile, *g\_Methyлотenera*, and *g\_Methylophilus* can further utilize one-carbon intermediates, such as methanol and formate,

produced during the degradation process (Idomeh et al., 2021; Jin et al., 2019a). This enables the microbial community to establish a complete metabolic chain from complex pollutants to small molecules and, ultimately, to mineralization.

### Figure 6.

### 3.6 Transition of the metabolism pathways

Metabolic pathways predicted using PICRUST2 further showed that basic metabolic pathways, including the TCA cycle, glycolysis, and amino acid synthesis, gradually decreased after integration with the ACF barrier and BF pretreatment process (Fig. 7a). A high abundance of basic metabolic pathways indicates a rapid-growth state in which large amounts of energy and amino acids are used for biomass accumulation, which usually contributes to severe biofouling. However, pathways related to the degradation of aromatic or complex organic compounds were significantly enhanced in the BF\_ACF system, indicating a stronger biodegradation capacity for refractory organics. This can directly promote the reduction of organic matter accumulated on the membrane surface and thereby alleviate membrane fouling. The relationships between dominant taxa in the UF and BF\_ACF systems and the related metabolic pathways (representing by the normalized abundance of the metabolism-related modules) were examined using Pearson correlation analysis (Fig. 7b). The results clearly showed that the abundances of dominant taxa in the UF system, including *g\_norank\_f\_Caldilineaceae*, *g\_norank\_f\_SM2D12*, *g\_norank\_f\_Micropepsaceae*, and *g\_Polynucleobacter*, were significantly positively correlated with basic metabolic pathways ( $r > 0.8$ ,  $p < 0.01$ ). In contrast, the two most abundant genera in the BF\_ACF system, *g\_Methylothermobacter* and *g\_Hydrogenophaga*, were significantly correlated with pathways related to refractory organic matter degradation ( $r > 0.8$ ,  $p < 0.01$ ). These findings demonstrate that the expression of different metabolic pathways is regulated by dominant taxa shaped by different operational regimes.

### Figure 7.

### 3.7 Discussion

Different regulation measures reshape the ecological strategies of microbial communities on the membrane surface by altering the composition of organics passing through the membrane and the associated environmental selection pressures, thereby determining the biofilm structure and membrane fouling trend (Fig. 8). In the UF system, the membrane surface is directly exposed to abundant readily biodegradable organics, promoting the dominance of fast-growing heterotrophs. As a result, fundamental growth-related metabolic pathways, such as glycolysis, the TCA cycle, and amino acid and nucleotide biosynthesis, are enhanced. The community therefore exhibits typical r-selected characteristics, namely rapid substrate utilization, rapid proliferation, and biomass accumulation, ultimately leading to a thicker and denser biofilm structure and severe membrane fouling. The ACF barrier in the ACF system reduces part of the organic load through adsorption and degradation, thereby alleviating fouling to some extent; however, its primary effect remains associated with physical retention/adsorption, and its ability to restructure microbial niches is limited. Therefore, it could be represented as a transitional state. In contrast, the BF\_ACF system (biofiltration pretreatment and ACF barrier) not only reduces readily biodegradable organics but also enriches functional taxa such as *Hydrogenophaga*, *Methylothermobacter*, *Methylophilus*, *Mycobacterium*, which are capable of aromatic compound degradation, one-carbon metabolism, stress resistance, and stable colonization. Correspondingly, functional modules related to benzene degradation, catechol cleavage, phenylacetate degradation, and formaldehyde assimilation are enhanced, whereas basic growth-related metabolism is relatively reduced. This indicates that BF\_ACF does not simply “reduce biomass”; rather, it transforms the membrane-surface ecosystem from a “fouling-type community dominated by rapid biomass accumulation” into a “cleaning-type community characterized by substrate mineralization, niche occupation, and stress-resistant stability.”

This mechanism has strong general applicability, as it essentially reflects a cascade regulation among substrate quality, ecological selection, community function, and membrane fouling behavior. Membrane fouling is not determined solely by microbial abundance, but more

importantly by the ecological strategies adopted by the microorganisms occupying the membrane surface. When readily biodegradable carbon sources are abundant, r-selected microbial populations can rapidly proliferate and cause high-biomass fouling. When biofiltration pretreatment is used this preferentially consumes readily biodegradable components, increases the complexity of residual organics, and provides continuous selection pressure, so that K-selected or functionally specialized populations become enriched. Although these microorganisms grow more slowly, they can utilize refractory substrates and prevent the recolonization of fouling-type heterotrophs through competitive exclusion. Therefore, BF\_ACF embodies an ecological engineering principle of “controlling fouling with microorganisms”: by artificially regulating the substrate spectrum and ecological niches, beneficial functional microorganisms are allowed to preferentially occupy the upstream/membrane-surface ecological space, thereby reducing foulant supply at the source and stably suppressing fouling-associated microbial populations. From a practical engineering perspective, the BF\_ACF system is particularly suitable for decentralized or rural applications, where low maintenance requirements and chemical-free operation are desirable. In addition, the system can operate solely under gravity-driven conditions, further enhancing its applicability and sustainability. The principle unveiled from this study can also be extended to other membrane-based water treatment systems. Thus, membrane fouling control should not rely solely on disinfection or physical cleaning, but should incorporate the targeted shaping of microbial community structure and metabolic function by upstream processes.

#### Figure 8.

## 4. Conclusion

This study has demonstrated that both the ACF barrier and the BF-integrated ACF barrier significantly improved the performance of the GDM system. Phenotypically, the biofilm formed in the BF\_ACF system was thinner and more porous, which facilitated water-channel formation and resulted in a higher stable flux. The selective enrichment of microbial communities driven by environmental organic matter was the fundamental mechanism

underlying this phenomenon. Compared with environments containing higher levels of biodegradable organic matter, biofilm formation under low-biodegradable-organic-matter conditions tends to rely more strongly on a K-selection-shaped community structure. This shift transforms the membrane-surface ecosystem from a “fouling-type community” into a “cleaning-type community.” This study reveals an ecological engineering principle by which pre-membrane substrate regulation drives niche transformation on the membrane surface and directionally shapes microbial community structure and metabolic functions. This principle is applicable not only to GDM systems but also to other membrane-based water treatment technologies, thereby improving their overall efficiency and sustainability.

#### **CRedit authorship contribution statement**

Lei Xu: Conceptualization, Supervision, Writing – original draft, review & editing; Qingqiang Dou: Investigation, Writing – review & editing; Nigel J. D. Graham: Writing – review & editing; Wenzheng Yu: Supervision, Writing – review & editing, Resources, Funding acquisition.

#### **Declaration of interests**

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.

#### **Data availability**

Data will be made available on request.

#### **Acknowledgments**

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#### **Appendix A. Supplementary data**

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## Figure Captions

**Figure 1.** Water quality characteristics during operation of GDM systems: (a) DOC concentrations of raw water, effluents of BF and different GDM systems; (b) Apparent MW distribution of DOM in raw water, effluents of BF and different GDM systems; (c-d) PARAFAC analysis and fluorescence indices of DOM in raw water, effluents of BF and different GDM systems: (c) fluorescence spectra of four DOM fractions; (d) Fmax values corresponding to the C1, C2, C3, and C4 fractions.

**Figure 2.** (a) Time-dependent membrane flux of different GDM systems, with the insert representing the average membrane flux at the steady-state; (b-c) THMFP and HAAFP of raw water, effluents of BF and different GDM systems; (d) Mantel test correlations between DBPFP, flux, and various water quality indicators ( $*p < 0.05$ ).

**Figure 3.** Surface morphology and cross-section structure of the biofilm formed on the UF membrane surface of the respective GDM systems: (a-c) UF system, (d-f) ACF system, and (g-i) BF\_ACF system; (j) content and (k) integral quantification of EEM fluorescence regions of microbially-derived biopolymers (represented by protein and polysaccharide) within the biofilm on the membrane surface of UF, ACF, and BF\_ACF systems; (l) water contact angles of air-dried fouled membranes of different GDM systems after long-term operation.

**Figure 4.** High-resolution C 1s (a-c) and O 1s (d-f) XPS survey spectra of air-dried fouled membranes of (a, d) UF, (b, e) ACF, and (c, f) BF\_ACF systems after long-term operation.

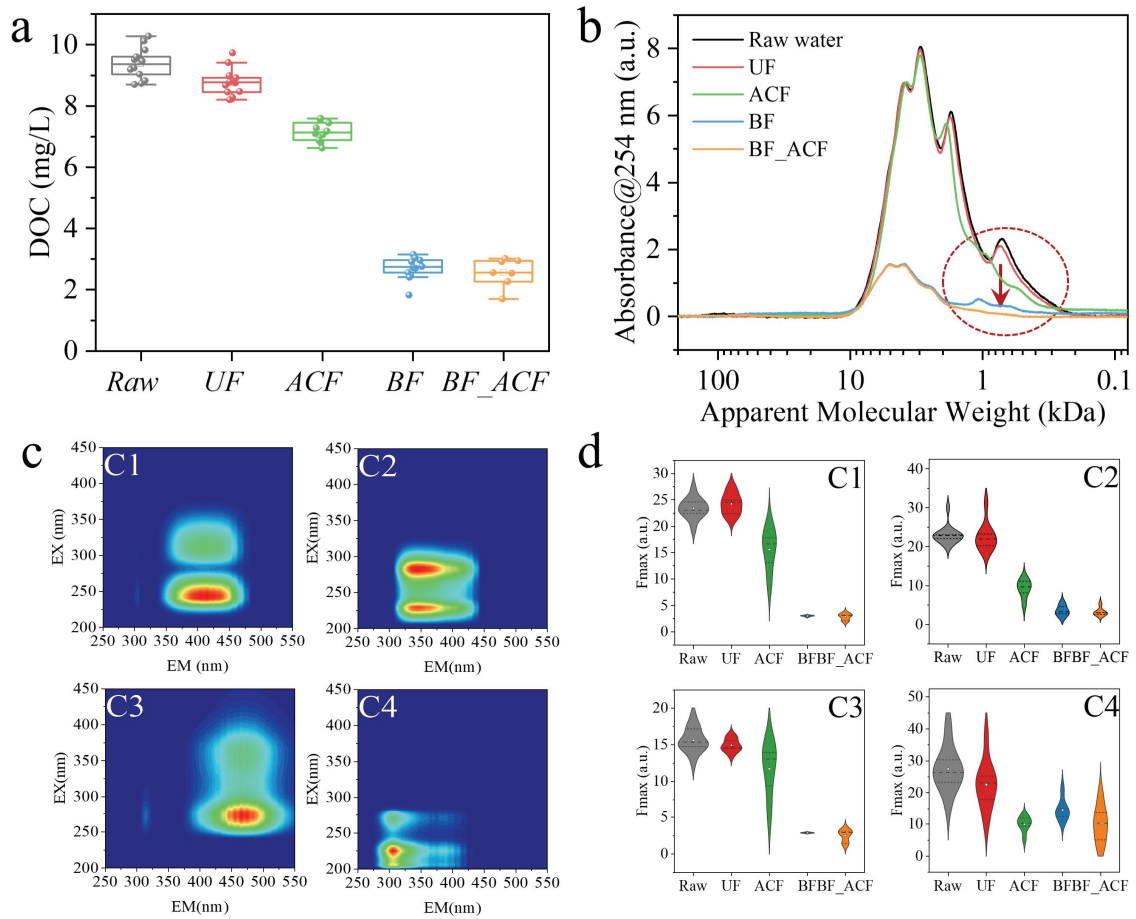
**Figure 5.** Microbial community diversity analysis: (a) Upset diagram showing shared and unique ASVs among the three systems; (b) Principal coordinate analysis (PCoA) plot based on Bray-Curtis dissimilarity of ASVs ( $r = 1$ ,  $p = 0.003$ ); (c-d) Alpha-diversity (Chao and Simpson indexes) of the microbial communities from the respective system on ASV level; (e-f) community similarity analyses based on different distance matrices: Bray-Curtis (e), weighted and unweighted UniFrac (f).

**Figure 6.** (a) Taxonomic composition of the microbial community within the biofilm formed on the membrane surface from the respective systems at the phylum level; (b) ternary

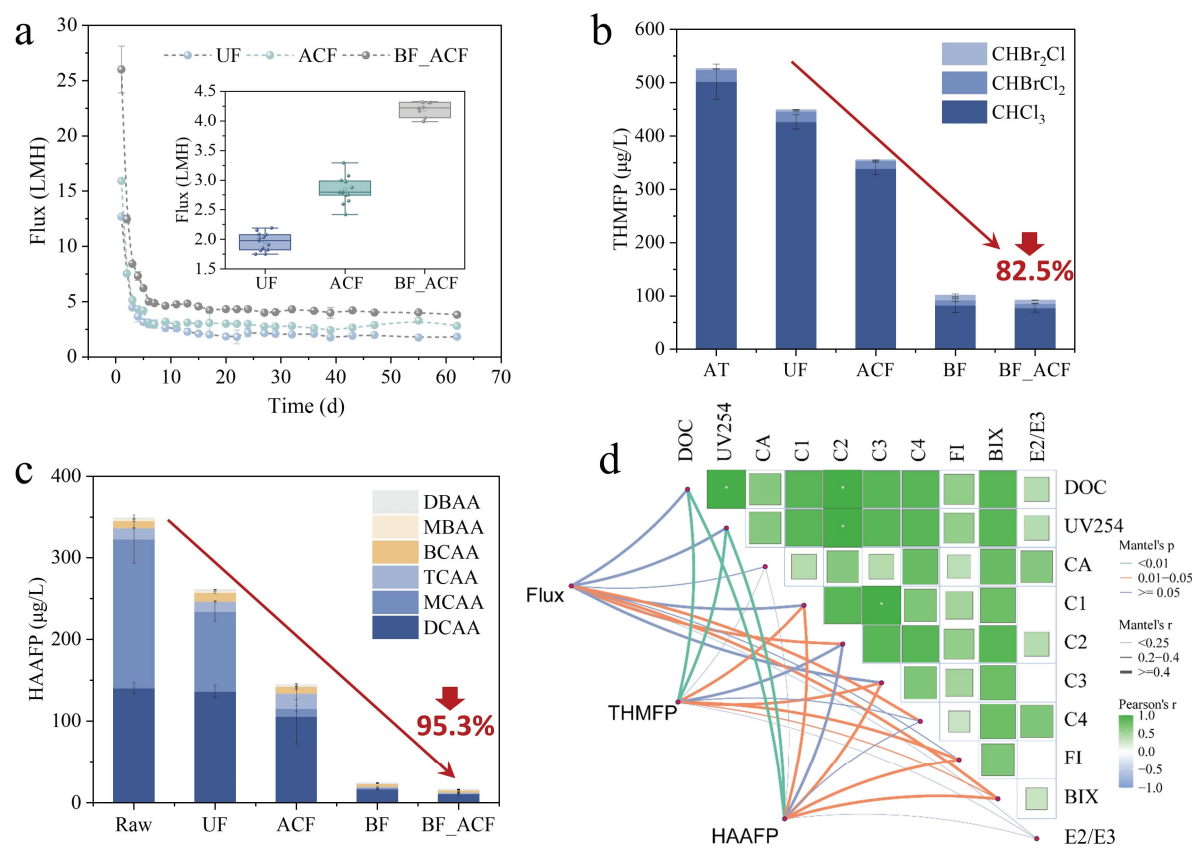
analysis of the microbial community structure at the order level, with color representing at the class level; (c) Heat map of the top abundant genera (top 30) enriched on the surface of the membrane from the respective systems (triplicate for each).

**Figure 7.** (a) Normalized abundance of the metabolism-related modules for basic metabolism pathways (including TCA cycle, glycolysis, and amino acid biosynthesis, etc.), and biodegradation pathways, especially for aromatic and complex organic matters, based on KEGG from different biofilm samples; (b) transition of the dominant microbial species between UF and BF\_ACF systems, and the Pearson analysis between the related metabolism pathways and the dominant species within UF and BF\_ACF systems ( $r > 0.8$ ,  $p < 0.01$ ).

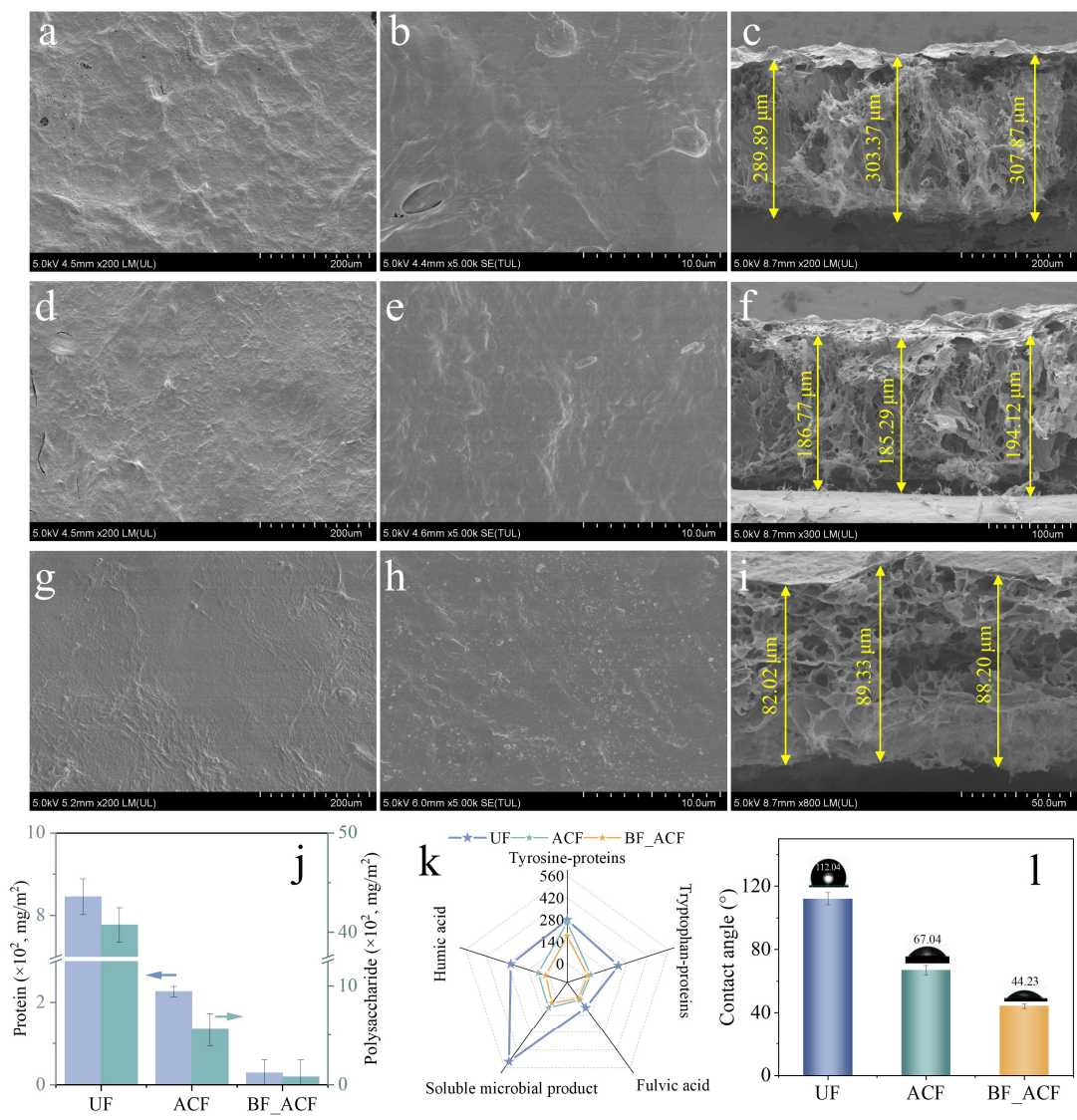
**Figure 8.** Schematic illustrating the effect of ACF barrier and BF integrated ACF barrier in regulating the biofilm formation on the membrane surface of GDM system.



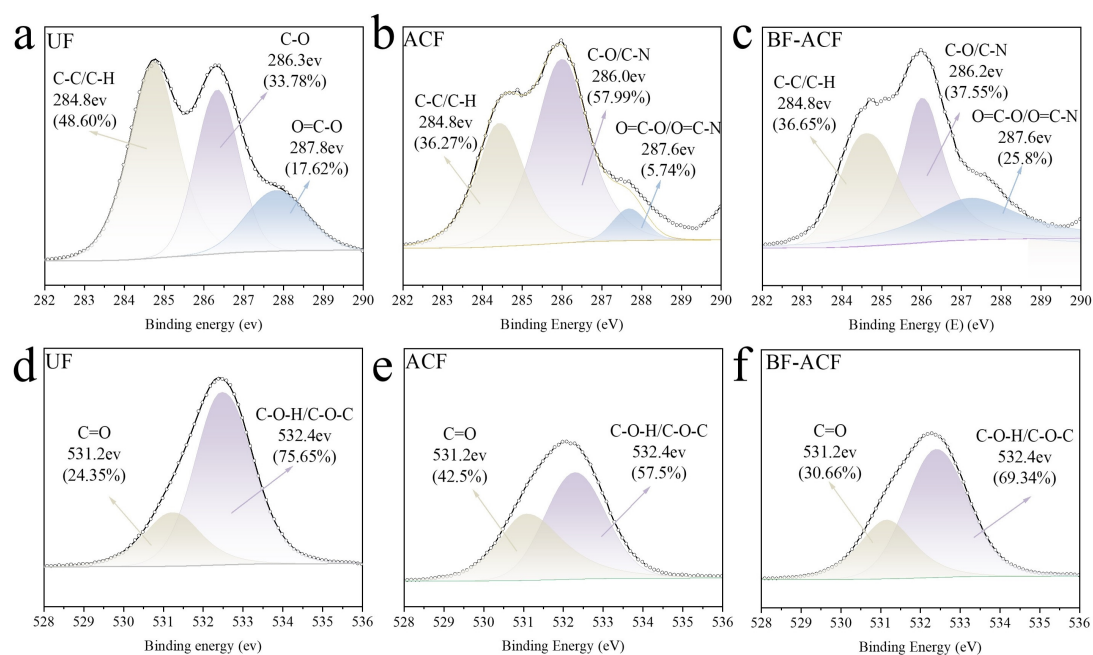
**Figure 1.** Water quality characteristics during operation of GDM systems: (a) DOC concentrations of raw water, effluents of BF and different GDM systems; (b) Apparent MW distribution of DOM in raw water, effluents of BF and different GDM systems; (c-d) PARAFAC analysis and fluorescence indices of DOM in raw water, effluents of BF and different GDM systems: (c) fluorescence spectra of four DOM fractions; (d) Fmax values corresponding to the C1, C2, C3, and C4 fractions.



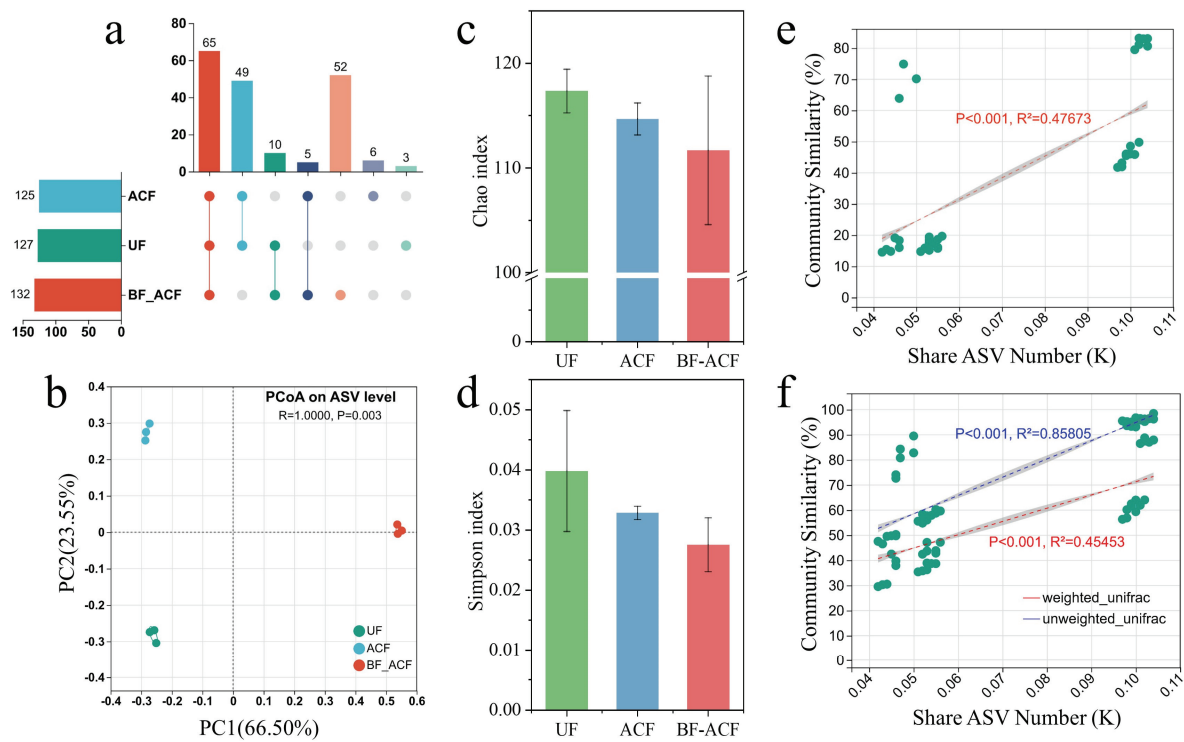
**Figure 2.** (a) Time-dependent membrane flux of different GDM systems, with the insert representing the average membrane flux at the steady-state; (b-c) THMFP and HAAFP of raw water, effluents of BF and different GDM systems; (d) Mantel test correlations between DBPFP, flux, and various water quality indicators (\*P < 0.05).



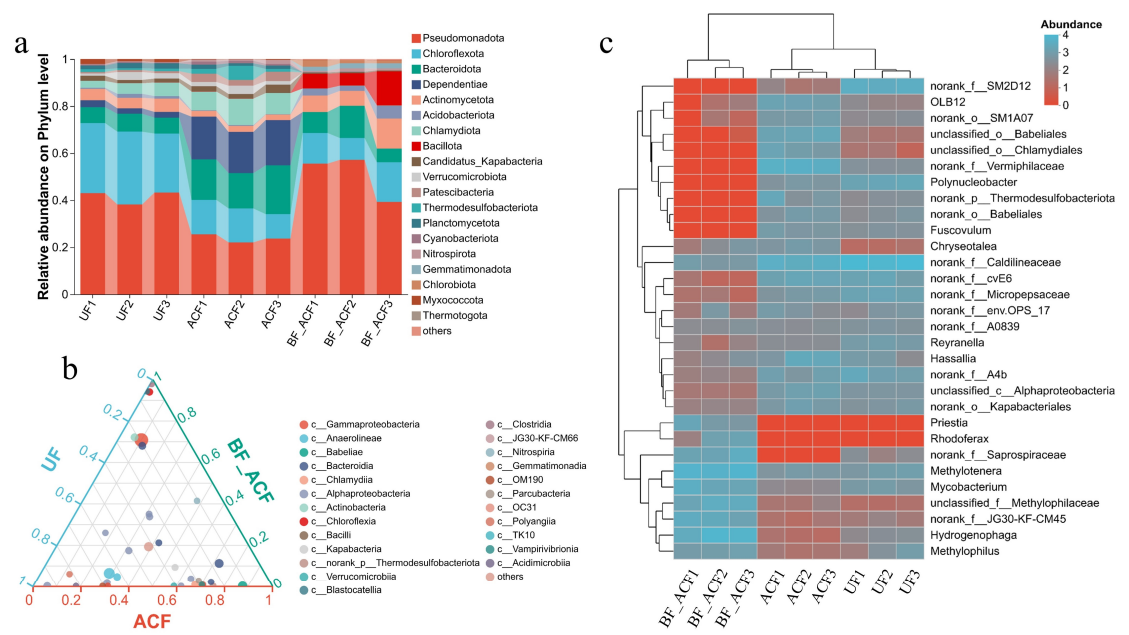
**Figure 3.** Surface morphology and cross-section structure of the biofilm formed on the UF membrane surface of the respective GDM systems: (a-c) UF system, (d-f) ACF system, and (g-i) BF\_ACF system; (j) content and (k) integral quantification of EEM fluorescence regions of microbially-derived biopolymers (represented by protein and polysaccharide) within the biofilm on the membrane surface of UF, ACF, and BF\_ACF systems; (l) water contact angles of air-dried fouled membranes of different GDM systems after long-term operation.



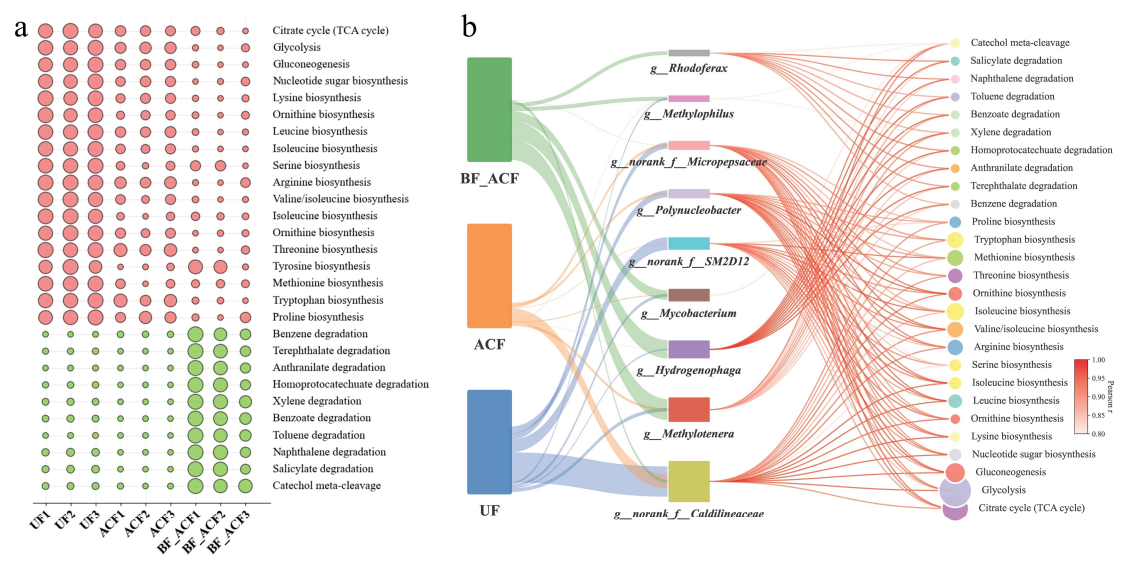
**Figure 4.** High-resolution C 1s (a-c) and O 1s (d-f) XPS survey spectra of air-dried fouled membranes of (a, d) UF, (b, e) ACF, and (c, f) BF\_ACF systems after long-term operation.



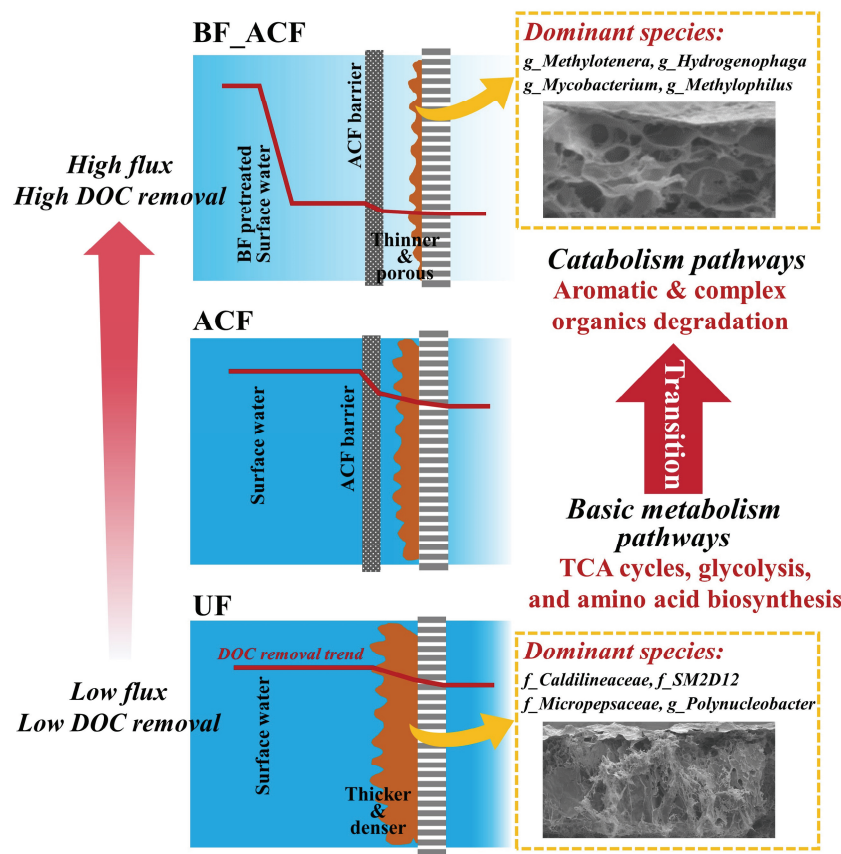
**Figure 5.** Microbial community diversity analysis: (a) Upset diagram showing shared and unique ASVs among the three systems; (b) Principal coordinate analysis (PCoA) plot based on Bray-Curtis dissimilarity of ASVs ( $r=1$ ,  $p=0.003$ ); (c-d) Alpha-diversity (Chao and Simpson indexes) of the microbial communities from the respective system on ASV level; (e-f) community similarity analyses based on different distance matrices: Bray-Curtis (e), weighted and unweighted UniFrac (f).



**Figure 6.** (a) Taxonomic composition of the microbial community within the biofilm formed on the membrane surface from the respective systems at the phylum level; (b) ternary analysis of the microbial community structure at the order level, with color representing at the class level; (c) Heat map of the top abundant genera (top 30) enriched on the surface of the membrane from the respective systems (triplicate for each).



**Figure 7.** (a) Normalized abundance of the metabolism-related modules for basic metabolism pathways (including TCA cycle, glycolysis, and amino acid biosynthesis, etc.), and biodegradation pathways, especially for aromatic and complex organic matters, based on KEGG from different biofilm samples; (b) transition of the dominant microbial species between UF and BF\_ACF systems, and the Pearson analysis between the related metabolism pathways and the dominant species within UF and BF\_ACF systems ( $r>0.8$ ,  $P<0.01$ ).



**Figure 8.** Schematic illustrating the effect of ACF barrier and BF integrated ACF barrier in regulating the biofilm formation on the membrane surface of GDM system.



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