

Long-term operation and biofouling of graphene oxide membrane in practical water treatment: insights from performance and biofilm characteristics

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Abstract

The widespread research of graphene oxide (GO) membranes has revealed the need to investigate their long-term performance and associated biofouling behavior in practical water treatment conditions. In this study, we have evaluated the long-term operation of GO membranes from the perspective of filtration performance and biofilm characteristics, based on two representative GO membranes (V-GO and P-GO, fabricated by vacuum and pressure filtration, respectively) with different surface properties and a natural surface water, using a gravity driven membrane system. The results showed that the GO membranes were capable of achieving sustainable water purification over 45 days of operation, associated with a complete rejection for biopolymers and desirable removal for fluorescent organic matters. Furthermore, the V-GO membrane with a rougher surface, reduced hydrophilicity and higher sheet-edge exposure than that of the P-GO membrane formed a highly heterogeneous and porous biofilm with high permeability, despite its greater biofilm thickness and extracellular polymeric substances (EPS) contents. In contrast, the P-GO membrane biofilm exhibited a thin but dense

structure, and therefore an increased hydraulic resistance (~1.5 times greater than V-GO membrane). The microbial community analysis revealed that bacteria related to biofilm formation was richer in V-GO biofilm. While bacteria associated with the degradation of organic matters and the aggregation of microorganisms accounted for a greater proportion in P-GO biofilm. These factors were responsible for forming a thick biofilm for V-GO, and a thin but high resistance biofilm for P-GO membrane. Our findings highlight the sustainable water purification performance of the GO membranes, and the P-GO membrane can alleviate biofilm formation but not necessarily reduce biofouling during the long-term filtration, while this was opposite in case of the V-GO membrane.

Key words: graphene oxide membrane; long-term operation; natural organic matter; biofilm structure; microbial community

1. Introduction

The lack of adequate access to clean and safe potable water continues to be a major threat to public health and the sustainability of modern society, due partly to excessive population growth and urbanization [1,2]. To ameliorate the problem of polluted water sources, there is an urgent need to develop advanced water treatment technologies to produce purified water from conventional and unconventional water resources [3]. Recently, membrane-based separation processes have been widely studied and used in drinking water production, wastewater reuse and desalination, as a consequence of their application versatility, excellent separation efficiency and relatively low cost [4,5]. However, the emergence of complex micro-pollutants, severe membrane fouling and the presence of corrosive chemicals in water are presenting considerable challenges to the performance of conventional polymer-based membranes [6]. Thus, the development of high selectivity, low fouling and chemically inert membrane materials, is urgently needed.

Graphene oxide (GO) membranes, which are assembled from individual GO sheets, have attracted enormous interest in terms of promoting progress in the evolution of membrane technology, owing to their unique advantages [7]. The nanochannels formed by the stacking of

GO nanosheets can provide an efficient water transport path, and a precise separation of target pollutants, based on size exclusion by the interlayer spacing. The rich oxygen-containing groups not only endow GO membranes with a superior hydrophilicity for preventing fouling, but also provide the potential for a variety of modifications to meet increasing separation applications [8]. With a decade of development, GO membranes have achieved various breakthroughs in understanding the mechanisms of separation and mass transfer, improving membrane permeability and selectivity, enhancing structural stability and large-scale fabrication [9-11]. Given these advances in the fundamental properties of GO membranes, there is a need to evaluate their performance in practical water treatment applications.

Natural organic matter (NOM), mainly composed of humic substances, polysaccharides and proteins, are commonly found in various natural water sources, and they are considered to be the significant factor affecting water quality and causing membrane fouling [12-14]. Several studies have shown that GO can effectively alleviate membrane fouling caused by NOM due to its excellent hydrophilicity, charge properties and oxidized edge channels [15,16]. These characteristics, taken together, weaken the adhesion of NOM on the GO surface. In addition to demonstrating outstanding NOM fouling resistance, research studies of GO membranes have also highlighted the superior removal performance of NOM [17,18]. GO membranes, suitably modified, are capable of almost completely removing representative organics, such as bovine serum albumin (proteins), humic acid (humic substances) and alginate (polysaccharides), and have exhibited a similar rejection performance to the commercial NF270 membrane for natural NOM, but the permeate flux was several times greater than that of commercial NF membranes [17,19]. These previous research studies highlighted the potential advantages of GO membranes for practical surface water treatment. However, the typically short filtration periods used, and the focus of using representative organic substances, in the aforementioned studies, were insufficient to assess the longer-term performance of GO membranes under conditions more realistic of water treatment processes. Considering the complexity of natural waters and the continuous deposition of pollutants in membrane surface, the sustainable performance of GO membranes, in terms of permeate flux, contaminant removal efficiency, performance

83 stability and biofouling, are still poorly understood, and requires detailed evaluation.

84 Pure GO membranes have been shown to readily disperse and peel off from the underlying
85 substrate in water environments [20]. Although substantial efforts have been made to
86 immobilize the GO layer and enhance the interfacial compatibility between the GO layer and
87 substrate [21,22], the weak secondary bonding is insufficient to allow the membrane to be
88 backwashed as frequently as with polymer membranes. Thus, the avoidance, or minimization,
89 of backwashing operations, is required for the practical application of GO membranes at
90 present. However, membranes inevitably suffer from biofouling when exposed to natural
91 waters (such as surface water, seawater) for a long duration [3]. Biofouling is defined as the
92 attachment of microorganisms to the membrane surface which results in the colonization and
93 growth of microbial cells, subsequently forming a matrix of microorganisms and their self-
94 produced EPS, known as a biofilm [23,24]. The formation of biofilm can be affected by many
95 factors, including membrane types [25], raw water properties [26], operating temperature [27],
96 pressure [28] and pretreatments [29]. These factors, in practice, change the composition of the
97 organic matters and microorganisms in biofilm, and thus affecting the formation and structure
98 of the biofilm. It has been reported that GO can induce microbial cell membrane damage by
99 physical puncturing, oxidative stress and phospholipid extraction [30,31], implying that the
100 GO has an antimicrobial potential which can reduce biofouling. In practice, the use of GO as a
101 functionalized layer to preclude the biofouling of commercial membranes has been verified in
102 recent studies [32,33]. These preliminary findings indicate that GO membranes have significant
103 promise for long-term operation. However, the formation of biofilm on the membrane surface
104 influences the permeation and separation performance during prolonged operation [34].
105 Therefore, identifying the characteristics of biofilms on the GO membrane surface is
106 fundamental to evaluate its long-duration performance, but investigations relevant to this have
107 not been reported until now.

108 Gravity driven membrane (GDM) filtration is a dead-end filtration system, which can
109 usually operate for several months without any backwashing [35]. GDM provides an
110 opportunity to explore the properties of biofilm as it provides a favorable environment for the

growth of a biofilm [36]. Therefore, in the light of the above, this study used GDM to investigate the long-term filtration performance and biofilm characteristics of GO membrane. The study was conducted over a 45 day duration based on two representative GO membranes with different surface microstructure and a natural surface water. Filtration performance and biofilm features were investigated rigorously. Moreover, the potential mechanism of the biofilm formation with different microstructures was specifically focused, together with the structure-performance relationship between biofilm characteristics and filtration performance.

2. Materials and methods

2.1. Fabrication and characterization of GO membranes

GO powder was synthesized by a modified Hummers method and the detailed process can be found in the previous studies [37]. The as-prepared GO powder was dispersed into DI water under continuous stirring and ultra-sonication to form a homogenous dispersion of 1 mg/mL. To obtain a uniform and stable GO membrane, Fe(III) (FeCl_3 , analytical reagent, Sinopharm Chemical Reagent Co., Ltd, China) was used as the crosslinking agent and the corresponding crosslinking procedure was performed by mixing the diluted GO dispersion with the same volume of Fe(III) (0.5 mmol/L). Subsequently, the mixed dispersion was run through a mixed cellulose ester supported membrane (Shanghai xinya purification equipment Co., Ltd, China, pore size: 0.22 μm) by vacuum and pressure filtration, respectively. The vacuum filtration was conducted using a dead-end filtration unit and the suction was provided by a circulating pump under a constant value of -1 bar. The pressure filtration was achieved by a filter cell (Millipore, Amicon 8400, USA) and the applied pressure (1 bar) was supplied by N_2 . Both membranes were prepared using the same GO loading of 0.4 g/m^2 . Finally, the resultant membranes were cleaned by passing DI water for several hours to remove the un-bonded Fe(III) and impurities. The prepared membranes were denoted as V-GO (vacuum filtration) and P-GO (pressure filtration).

The physicochemical properties of the GO membranes were analyzed by a variety of techniques. Scanning electron microscopy (SEM, JSM-7001F+INCA X-MAX, Japan) was

used to provide the morphology information of the membrane. Before the measurement, the membrane samples were pre-coated with a thin platinum layer for 120 s to enhance the conductivity of the samples. Atomic force microscopy (AFM, Fastscan Bruker, USA) was applied to reveal the surface roughness of the membranes. Three randomized locations were selected and averaged for the calculation of roughness. Attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR, Spectrum Two, PerkinElmer, USA), with a wavenumber range of 4000-400 cm^{-1} , was performed to identify the surface functional groups of the membranes. X-ray diffractometer (XRD, Rigaku SmartLab 9 kW, Japan) was used to analyze the interlayer spacing of the membranes. The hydrophilicity of the membranes was quantified by using a contact angle goniometer (WCA, OCA15EC, Dataphysics, Germany). The membrane samples were cut into strips and fixed on the edge of a slide. A 2 μL quantity of DI water was dropped on the surface of samples and the recording time was ~ 180 s. At least three randomized locations were selected and averaged for the measurement.

2.2. Raw water and experimental setup

Surface water, sampled from the river Jingmi (Beijing, China), which is one of the important sources of drinking water supply for Beijing, was used as the raw water for this study. A sufficient quantity of sample was collected and immediately filtered by filter paper (qualitative, 102, Hangzhou Special Paper Industry Co., Ltd, China) to remove particulate impurities and then stored in a cold storage at ~ 4 $^{\circ}\text{C}$. The basic characteristics of the raw water can be found in the Supporting Information (SI, Table S1). Before the GDM test, the water samples need to be restored to room temperature at ~ 21 $^{\circ}\text{C}$. Raw water is supplemented and refreshed at the initial day and day 22 of the experiment.

The experiment was carried out in a bench-scale GDM system and a schematic diagram of the system is shown in Figure S1. Specifically, the two types of GO membranes were assembled in two parallel dead-end membrane modules with an effective filtration area of 12.56 cm^2 . A constant-level tank was employed to distribute raw water to each membrane module and the vertical distance between membrane surface and the water level of the constant tank was 60 cm (providing a transmembrane pressure: 60 mbar). A high-level tank was connected

to the level controller of the constant-level tank to continuously supply raw water and balance the water level. The GDM system was continuously operated for 46 days at a near-constant room temperature (at 21 ± 2 °C).

2.3. Permeate flux and filtration resistance

The permeate flux was tested by recording the weight of the permeate water at 1 h intervals once a day and then calculated based on Eq. 1, where J ($\text{L m}^{-2} \text{h}^{-1}$) denotes the permeate flux, V (L) represents the volume of permeated water, A (m^2) is the effective filtration area and ΔT (h) is the filtration time.

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

The total resistance R_t (m^{-1}) was calculated according to Eq. 2, where TMP (Pa) is the transmembrane pressure, μ ($\text{Pa} \cdot \text{s}$) is the dynamic viscosity of water at the test temperature.

$$R_t = \frac{TMP}{\mu \cdot J} \quad (2)$$

The total resistance can be represented as the sum of the intrinsic membrane resistance and biofilm resistance. Thus, the biofilm resistance R_b (m^{-1}) was calculated according to Eq. 3 [38], where R_m (m^{-1}) is the intrinsic membrane resistance and the corresponding calculation is similar to Eq. 2, except that J represents the pure water flux of the membrane under the operation water height.

$$R_b = R_t - R_m \quad (3)$$

2.4. Organic matter analysis

The organic matter in water was analyzed, qualitatively and quantitatively, using several spectroscopic and chromatographic techniques. Ultraviolet spectrophotometry (UV-2600, Shimadzu, Japan) was employed to determine the specific absorption at a wavelength of 254 nm (UV_{254}) to represent the concentration of organic matter. Three-dimensional excitation-emission matrix fluorescence spectroscopy (EEM, F-4600, Hitachi, Japan) was carried out to identify the different fluorescent compounds, such as aromatic protein-like or humic acid-like substances. The scan ranges were 200-550 nm for emission and 200-450 nm for excitation wavelengths. The PMT voltage was maintained at 500 V and the scan speed was 2400 nm/min.

In addition to qualitative identification, the quantitative method of parallel factor analysis (PARAFAC), was performed to determine the content of each fluorescent compound [39]. The apparent molecular weight (MW) distribution of organic matter was determined by high-performance liquid size exclusion chromatography (SEC). SEC was performed using a Waters HPLC system with the following components: a SEC column (Phenomenex, BioSep 5 μ m, SEC-s3000, 290Å), a security guard column (Phenomenex, KJ0-4282), a 1525 binary pump, 1500 column heater and auto-sampler. 10 mM of sodium acetate (Aldrich, USA) was used as the mobile phase and the flow rate was maintained at 1 mL/min. Before the SEC test, all samples were pretreated by 0.45 μ m filter.

2.5. Biofilm analysis

At the end of the GDM process, the membranes were taken out from the modules and cut into several parts for biofilm analysis. The EPS was extracted from the membrane surface for the analysis of polysaccharide and protein contents. The extraction method was based on a sequential vortex, heating and centrifugation. In brief, one-fifth of the fouled membrane samples were first cut into pieces and suspended in 4 mL of phosphate buffer solution (PBS) with continuous vortex for 10 min. After vortex, the pieces were taken out and the remaining suspension was transferred to a water bath and heated at 80 °C for 30 min. Then, the suspension was centrifuged at 10000 r/min for 10 min and the supernatant was collected for subsequent analysis. The contents of polysaccharide and protein were analyzed according to the phenol sulfuric acid method using glucose as the standard and the Bradford method with bovine serum albumin as the standard, respectively [40,41].

SEM was performed to reveal the surface and cross-sectional morphology of biofilms. To preserve the pristine structure, the fresh biofilms were immediately frozen at -50°, followed by freeze-drying for SEM observation. The functional groups of biofilms were investigated by ATR-FTIR and the data were analyzed after baseline correction and normalization. X-ray photoelectron spectroscopy (XPS, 250Xi, Thermo Scientific, USA) provided information about the chemical bonds and element content of the biofilm. The hydrophilicity of biofilms was evaluated using a contact angle goniometer. All instrument information and operating

parameters are consistent with section 2.1 unless otherwise specified.

Confocal laser scanning microscopy (CLSM) can provide a microscopic visual distribution of eDNA, proteins and polysaccharides within biofilms. The fresh biofilms were first fixed with 2.5% formaldehyde solution for 30 min and then transferred to a dark environment for staining. Fluorescein isothiocyanate (FITC, Sigma-Aldrich, USA), Concanavalin A (ConA, Sigma-Aldrich, USA) and 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, USA) were sequentially used to stain proteins, α -mannopyranosyl and α -glucopyranosyl sugar residues, and eDNAs, respectively. Each staining process lasted for 30 min and was followed by washing with phosphate buffer solution (PBS) 5 times. After staining, the samples were assembled on a confocal laser petri dish and visualized by CLSM (TCS SP5, Leica, Germany).

2.6. Microbial community analysis

At the end of the membrane operation, the fresh biofilms were carefully removed and immediately transferred to a petri dish at -20 °C before microbial community analysis. The genomic DNA was extracted from the biofilms using the E.Z.N.A. ® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) based on the manufacturer's instructions. The 16S rRNA gene was amplified with primer pairs 338F and 806R using a PCR amplifier (ABI GeneAmp® 9700, ABI, USA). After extraction and amplification, the PCR products obtained were treated by purification, quantification and then sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA). The UPARSE pipeline was employed to cluster the operational taxonomic units (OTUs) with 97% similarity cutoff, and chimeric sequences were identified and removed. The taxonomic classification of each OTU representative sequence was performed using a Ribosomal Database Project (RDP) classifier against the Silva v138/16s database using confidence threshold of 0.7. The data presented in this study were all analyzed and obtained through the online platform of Majorbio Cloud Platform (www.majorbio.com).

3. Results and discussion

3.1. Characterizations of GO membranes

SEM images indicated that the prepared GO membranes were uniform and defect-free, as well as representative wrinkles can be observed on the membrane surface (Figure 1a). The edge exposure of GO nanosheets could be clearly observed on the V-GO membrane surface, while no obvious nanosheet edges were found on the P-GO membrane (inset of Figure 1a). AFM results showed that the average roughness (Ra) of P-GO membrane was 92.6 nm, which was lower than that of the V-GO membrane (99.2 nm) (Figure 1b). The higher roughness of V-GO can be attributed to the greater wrinkle height. From the measurements of water contact angle (Figure 1c), the V-GO membrane exhibited a higher contact angle (59.7°) than that of the P-GO membrane (53.4°), indicating a greater hydrophilicity of the P-GO membrane. The results of contact angles and roughness were consistent with the previous finding that GO membranes prepared by vacuum filtration exhibited greater roughness and contact angle than that of the membrane prepared by pressure filtration [42]. However, the higher surface roughness accompanied with a greater contact angle of GO membrane seem to be different from the opinion that a rougher surface had a lower contact angle for a hydrophilic material [43]. This can be attributed to the following reasons: GO is essentially an amphiphilic material with hydrophobic base plane and hydrophilic oxidation regions. Compared with P-GO membrane, V-GO membrane exhibited a greater roughness and wrinkle height (the vertical distance between ridges and valleys). When water contacts with the GO membrane surface, water first tends to accumulate on the space between the two ridges/peaks of the GO wrinkles, and then spreads horizontally. However, due to the increase of the wrinkle height on the V-GO membrane surface, the resistance of water to spread horizontally increases, making water more difficult to reach the next wrinkle during the spreading, and therefore a higher contact angle [44]. The FTIR spectra for the two membranes showed that the chemical groups of the two membranes were the same, including -OH, C=O, C=C, C-OH, C-O-C and C-O bonds, over the wavenumber range of $4000\text{-}1000\text{ cm}^{-1}$, which is consistent with previous reports [45] (Figure 1d). The XRD patterns of the membrane showed that the diffraction peak of V-GO located at

$2\theta = 10.24^\circ$, corresponding to interlayer spacing of 0.86 nm (Figure S2). While the interlayer spacing of P-GO membrane slightly decreased to 0.84 nm, indicating that P-GO membrane has a more compact structure. In addition, the full-width half-maximum of P-GO membrane (1.25) was narrower than that of the V-GO membrane (1.69), indicating that the stacking of GO nanosheets in P-GO membrane was more order than V-GO membrane [42].

Figure 1

3.2. Permeate flux and biofilm resistance

The filtration performance and biofilm characteristics were evaluated using a bench-scale GDM setup (Figure S1). The permeate flux over 45 days of operation is presented in Figure 2a. Specifically, the permeate flux varied with time and broadly divided into two stages, stage I (days 0-24) and stage II (days 25-46). In the initial stage, the permeate flux of P-GO ($2.7 \text{ L m}^{-2} \text{ h}^{-1}$) was slightly lower than that of the V-GO ($3.4 \text{ L m}^{-2} \text{ h}^{-1}$) due to the more compact structure caused by the pressurized assembly. Then, the permeate flux experienced a continuous reduction to approximately 1.2 and $0.9 \text{ L m}^{-2} \text{ h}^{-1}$ for V-GO and P-GO, respectively, during the stage I. The considerable flux decrease was the consequence of nanochannel blocking and the formation of biofilm on the membrane surface. In the following stage II, the permeate flux stabilized at a certain value until the end of the operation. The permeate flux of V-GO was stable at $\sim 1.2 \text{ L m}^{-2} \text{ h}^{-1}$, while the P-GO was only $\sim 0.8 \text{ L m}^{-2} \text{ h}^{-1}$ (inset of Figure 2a). The stable flux is the main character of GDM filtration and this phenomenon is associated with a heterogeneous structure of biofilm with considerable cavities and channels, which decreases the hydraulic resistance and balances the permeate flux [26].

The hydraulic resistance was analyzed as shown in Figures 2b and 2c. Similar to the permeate flux, the development of biofilm hydraulic resistance displayed a continuous increase before reaching a relatively stable stage. During the initial 7 days, the increase in resistance for both membranes was similar, while in the subsequent period of operation, the resistance of the V-GO membrane increased slowly and then stabilized at $\sim 13 \times 10^{12} \text{ m}^{-1}$ after 46 days operation

(Figure 2b), which accounted for 65% of the total hydraulic resistance (Figure 2c). In contrast, the resistance of the P-GO membrane continued to increase rapidly and reached a higher resistance value of $\sim 19 \times 10^{12} \text{ m}^{-1}$, which represented 70% of the total hydraulic resistance. It is worth noting that flux of V-GO membrane decreases more, but the biofilm resistance is lower compared with P-GO membrane. This is mainly due to the non-linear variation of flux with biofilm resistance (Eq.2 and Eq.3) in GDM system. Figure S4 showed the variation of flux with biofilm resistance for V-GO and P-GO, it can be seen that at the high flux stage (the initial operation stage), the increase of biofilm resistance corresponds to a high decrease of flux. While at the low flux stage, the same resistance increase corresponds to a significantly lower flux decrease. Although the flux of V-GO decreases more during the operating period, the stable flux of V-GO ($1.2 \text{ L m}^{-2} \text{ h}^{-1}$) is higher than that of P-GO ($0.8 \text{ L m}^{-2} \text{ h}^{-1}$). The flux decreases from 1.2 to $0.8 \text{ L m}^{-2} \text{ h}^{-1}$ needing a much higher biofilm resistance as seen in Figure S4. Thus, the biofilm resistance of V-GO is lower than that of P-GO membrane. In addition, the biofilm resistance of P-GO membrane appeared to show a large fluctuation after 25 days. This may be related to the more sensitivity of microorganisms in P-GO biofilm to the variation of water environment, since the feed water was refreshed and supplemented at day 22 of the experiment. The quality of the newly supplemented water is slightly different from the water that already existed in the GDM system, which may affect the microbial activity and hence the fluctuation of biofilm resistance. Together, the results of the permeate flux and biofilm resistance indicated that the structure of biofilms on the V-GO and P-GO membrane surfaces were different and that the V-GO surface formed a more permeable biofilm than that of the P-GO surface. Moreover, the lower biofilm resistance also demonstrated that the V-GO membrane performs better in alleviating biofouling compared to P-GO membrane.

3.3. Removal of organic matter

EEM spectra can identify fluorescent organic matter in water, such as aromatic protein-like and humic substances. The results given in Figure 2d showed that the fluorescent organics in the raw water comprised aromatic protein-like (regions I and II), soluble microbial by

product-like (SMP, region IV), and humic-like substances (regions III and V). After filtration by GO membranes, the fluorescence intensity of aromatic protein-like and SMP-like substances clearly decreased, indicating that the membranes preferentially removed both components. The results of PARAFAC analysis further showed that V-GO and P-GO membranes achieved favorable and similar removal for the C2 component (associated with protein-like substances [46]) with corresponding values of 61% and 64%, respectively (Figures S6 and S7). The fluorescence intensity of humic substances (region III: fulvic acid-like and region V: humic acid-like substances) in the feed and filtrate waters were almost unchanged and independent of the type of GO membranes. The MW distributions of feed and filtrate waters were determined by SEC. In general, the organic matter with MW distribution of 30-80 kDa, 1-10 kDa and 800 Da were related to biopolymers (such as polysaccharides and proteins), humic acid and the low MW organic matter associated with biological activity, respectively [47,48]. It was clear that the biopolymers could be completely removed by both membranes, while the other components were partly removed and both membranes performed better in removing the biological activity substances compared to humic substances (Figure 2e). In addition, the removal of organic matter was also indicated by the lower UV₂₅₄, SUVA and higher E₂/E₃ (the ratio of UV absorbance at 254 to 365 nm) in the filtrate waters (Figure S8).

The stable permeate flux and organics removal confirmed that the GO membranes were able to achieve sustainable water purification under long-term operation. In addition, the highly analogous FTIR spectra of the pristine and the fouled membrane (after rinsing by DI water) indicated that no chemical damage occurred to the GO membrane during the operation (Figure S9).

Figure 2

3.4. Properties of biofilms

3.4.1. Morphologies and structures of biofilms

The stable permeate flux and hydraulic resistance were mainly governed by the

morphologies and structure of the biofilms. SEM images showed that the morphologies of biofilms on the V-GO and P-GO membranes exhibited significant differences. Specifically, the biofilm formed on the V-GO surface showed a loose and porous structure, and the network structure inside the biofilm had more void space (Figure 3a). In contrast, for the P-GO biofilm, although the presence of pores between patches of biofilm, the pore size was smaller than that of the V-GO and the structure appeared to be smoother and denser (Figure 3b). Cross-sectional SEM images further showed that the biofilm formed on the V-GO surface had a greater thickness of $\sim 1.90\ \mu\text{m}$, compared to that of the P-GO biofilm ($\sim 1.46\ \mu\text{m}$) (Figures 3c and 3d), indicating that more EPS accumulated on the membrane surface. Furthermore, the biofilm on the V-GO surface displayed a highly heterogeneous and porous structure, while the P-GO biofilm exhibited a dense lamellar structure with less voids. Previous studies have shown that the structure of biofilm was affected by the bacterial growth and the movement and predation of eukaryotes in the biofilm [49]. Thus, the highly heterogeneous and porous structure of the V-GO biofilm was probably related to more movement and predation of eukaryotes, and the specific bacterial community.

The permeate flux and hydraulic resistance were consistent with the micro-visual observations of the biofilms structure. For the V-GO biofilm, the porous structure contributes to a high permeate flux and low hydraulic resistance. In contrast, a dense structure of P-GO biofilm result in a high hydraulic resistance. It was also notable that the thickness of the biofilm formed on the V-GO was greater than that of P-GO, while its hydraulic resistance was significantly lower. These results highlight that the hydraulic resistance was strongly associated with the structural nature of biofilm, such as their porosity, rather than the thickness.

The distribution of eDNA, proteins and polysaccharides in the biofilms were visualized by CLSM images (Figures 3e-l). The fluorescent intensity of eDNA (blue) and polysaccharide (red) in the V-GO biofilm were stronger than that of the P-GO, indicating that there was a greater presence of microorganisms and polysaccharides in the V-GO biofilm. Furthermore, more algae were observed in the P-GO biofilm and they were the major contributor to the fluorescent signals. In comparison, for the V-GO biofilm, the number of algae was less than

that of the P-GO, and the uniform distribution of eDNA and the large size protein cluster demonstrated the different biofilm constituents between the V-GO and P-GO. Previous studies have shown that more edge exposure of GO nanosheets could enhance algal cell death by the maximization of edge mediated interaction [50]. Tsou et al. found that a GO membrane prepared by vacuum filtration exhibited a higher edge exposure than that of a pressure-filtration membrane [42]. SEM images also provided evidence of the enhanced edge exposure of GO nanosheets on the V-GO membrane surface. Thus, the growth of algae cells on the V-GO membrane was probably suppressed due to the higher physical damage (puncture). Moreover, the presence of algae usually leads to an increased fouling resistance and reduced microbial activity in GDM systems [29]. Therefore, the lower algae content in the V-GO biofilm would contribute to the formation of a heterogeneous and more permeable biofilm, which is consistent with the results of Section 3.2.

Figure 3

3.4.2. Physicochemical properties of biofilms

The thicker biofilm and stronger eDNA and polysaccharide fluorescence signals suggested more EPS was present in the V-GO surface, and this was confirmed by the higher content of proteins and polysaccharides extracted from the V-GO than that of the P-GO biofilm (Figure 4a). The MW distribution of organic matters in biofilms displayed three regions at 100-1000 Da, 1-10 kDa and 10-100 kDa, related to low MW substances, humic substances and biopolymer, respectively (Figure 4b). The higher intensities of biopolymer and humic substances in the V-GO biofilm than that of the P-GO implied that more proteins, polysaccharides and humic substances were present. The water contact angle of the fouled membranes revealed the hydrophilicity/hydrophobicity of the biofilms. In contrast to the virgin GO membranes, the contact angle of the fouled membranes significantly increased to 98.2° for the V-GO and 94.6° for P-GO, indicating that the developed biofilms had a high hydrophobicity (Figure 4c). The slightly higher contact angle of the V-GO biofilm compared to the P-GO

initially suggested that there were more hydrophobic substances present in the V-GO biofilm. Although being more hydrophobic, the contact angle of V-GO biofilm exhibited a rapid and continuous decline within 180 s, while the contact angle of the P-GO biofilm decreased more gradually and reached a relatively stable value at the end of the measurement record. The rapid decline rate of contact angle is associated with porous structure [51,52], which is consistent with the SEM results. The chemical composition of biofilms were analyzed using ATR-FTIR spectroscopy (Figure 4d). Both V-GO and P-GO biofilms showed similar peaks at the wavenumbers of 1000-4000 cm^{-1} , including 3280 cm^{-1} (O-H or N-H), 2922 and 2852 cm^{-1} (C-H), 1724 cm^{-1} (C=O from carboxyl), 1635 cm^{-1} (C=O from amide), 1543 cm^{-1} (N-H or C-N from amide), 1242 cm^{-1} (C-O from carboxyl) and 1044 cm^{-1} (C-O from aliphatic component). These functional groups, in practice, were mainly derived from the proteins, polysaccharides and humic substances in biofilms, as reported previously [53,54]. The peak intensities at 3280 cm^{-1} , 1635 cm^{-1} and 1543 cm^{-1} in the V-GO biofilm were visually stronger than those of the P-GO biofilm, further confirming more EPS content in the V-GO biofilm.

Figure 4

XPS spectra provides further information about the composition and proportion of chemical bonds of biofilms. Figure 5 displayed the high resolution C1s, O1s and N1s XPS spectra. The C1s peak was resolved into three Gaussian peaks at binding energies of 284.8, 286.5 and 288.0 eV, and the corresponding chemical bonds were C-C/C-H, C-O/C-N and O=C-O/O=C-N, respectively [48]. C-C/C-H was related to aliphatic and aromatic hydrocarbon backbones, polysaccharides, amino acid side chains and lipids [48]; C-O/C-N was associated with alcohols, phenols, ester and amine [55]; O=C-O/O=C-N can be attributed to carboxylic acid, ester and peptide carbons from protein [36,56]. O1s can be decomposed into O=C-N/O=C-O (531.7 eV), O-C/O-H (532.7 eV), and O=C-O (534.1 eV) [55]. O=C-N/O=C-O and O=C-O were regarded as carboxylates, ester and amide from protein; O-C/O-H can be ascribed to alcohols, hydroxide and (hemi) acetals [55]. N1s at binding energies of 399.7 eV and 400.3

eV were assigned to N-H and C-N from amine, respectively [48], and the peak located at 408.3 eV belonged to the N-O from the nitro-compounds [57]. It was noted that the proportion of hydrophilic chemical bonds, including O-H and N-H in the P-GO biofilm (47% and 53%, respectively) were significantly greater than those in the V-GO biofilm (38% and 35%, respectively). This result was likely caused by the different microorganisms and their associated secretory products. Moreover, the higher hydrophilic compositions in the P-GO biofilm also contributed to the formation of a denser biofilm, since the hydrophilic content is more conducive to the aggregation of bacteria [58].

Figure 5

3.5. Analysis of microbial community

The microbial community within biofilms was analyzed according to high throughput sequencing. The high coverage index (>0.999) suggested that the most microbial communities present in both biofilms had been well captured (Figure S11). A Venn diagram showed that the OTUs in the V-GO and P-GO biofilms were 626 and 586, respectively, with 481 shared OTUs (Figure 6a). The differences of OTUs between the two biofilms not only suggested that the different microstructures of GO membranes affected the microbial diversity, but the higher number of shared OTUs also demonstrated that the different microstructures of the GO membranes mainly affected partial microbial community rather than cause the significant variation of the overall community. Furthermore, the biofilm formed on the V-GO surface had a higher microbial community richness than that of the P-GO, while the diversity was lower than the P-GO, as confirmed by the higher Chao index and lower Shannon index of the V-GO (Figure 6b). These results indicated that the V-GO membrane, with a rougher surface, reduced hydrophilicity and higher sheet-edge exposure, provided a particular environment that could only selectively tolerate the specific microorganisms to survive and increase their richness.

The dominant phyla bacteria in both V-GO and P-GO biofilms were assigned to Proteobacteria, Bacteroidota and Verrucomicrobiota, accounting for over 93% of the total

abundance (Figure S12). The phylum Proteobacteria accounted for 50% in the V-GO biofilm, followed by Bacteroidota (30%) and Verrucomicrobiota (14%). In comparison, the relative abundance of Proteobacteria and Bacteroidota decreased to 46% and 21%, respectively, and the Verrucomicrobiota increased to 26%, in the P-GO biofilm. At a class level (Figure 6c), the distinctions were greater. Specifically, Gammaproteobacteria and Bacteroidia showed greater abundance in the V-GO biofilm (34% and 30%, respectively) than that in the P-GO (24% and 21%, respectively). Bacteria Alphaproteobacteria, which has been recognized as the primary colonizers of the membrane surface accounted for 17% in the V-GO biofilm, but increased to 22% in the P-GO biofilm [59]. While bacteria Gammaproteobacteria, which has also been reported to be involved in bacterial colonization in the initial stage and formation of biofilm in the V-GO biofilm (34%) was significantly greater than the P-GO (24%) [60]. This result indicated that Gammaproteobacteria is preferred to colonize the V-GO surface, while Alphaproteobacteria is favored to colonize the P-GO surface. The initial colonization of bacteria on the membrane surface can be affected by the hydrophilicity of the membrane [61]. The previous study has also found that the relative abundance of Gammaproteobacteria increased with an increasing hydrophobicity of the membrane [62]. Thus, the different colonization behaviors of Gammaproteobacteria and Alphaproteobacteria on the V-GO and P-GO membrane surfaces are probably influenced by the surface hydrophilicity of the membrane. The difference in the primary colonizers represents an important factor causing the formation of different biofilms between the V-GO and P-GO surface. In addition, the relative abundance of Verrucomicrobiae in the V-GO (14%) was approximately half of that in the P-GO (26%). In comparison, Bacteroidia represented a higher proportion of 30% in the V-GO, and this value decreased to 21% in the P-GO biofilm.

At a genus level (Figure 6d), *Legionella* and a *norank_f_Diplorickettsiaceae*, both belong to class Gammaproteobacteria, showed a higher proportion in V-GO (28% and 2.8%, respectively) than that in the P-GO biofilm (17%, and less than 0.1%, respectively). Gammaproteobacteria has the capacity to secrete larger EPS [63], and therefore, the higher abundance of *Legionella* and *Diplorickettsiaceae* in V-GO biofilm resulted in the greater

content of proteins and polysaccharides. *A norank_f_Microscillaceae* with the capacity to feed species in the biofilms by secreting metabolic products as nutrition in an oligotrophic environment also accounted for a greater proportion in the V-GO (25%), compared to the P-GO (18%) biofilms [64]. The JM water used in this study was comparatively oligotrophic and the more *Microscillaceae* in the V-GO biofilm could provide a habitable environment for the inner-layer species to survive and motility. *Prostheco bacter* was the most abundant genus in the P-GO biofilms (26%) and the proportion was two-fold higher than that of the V-GO biofilm (13%). *Prostheco bacter* was associated with the EPS consumption, degradation of organic compounds and the denitrification under anoxic conditions [65,66]. Its increase in the P-GO biofilm was believed to favor the mitigation of biofilm formation, reduction of the organics content and nitro-compounds. Some bacterial genera, such as *Methylibium* and *Brevundimonas*, with the abilities to degrade aromatic matter, also exhibited a greater abundance in the P-GO biofilm [67,68], indicating that the presence of the above bacteria was beneficial to the mitigation of biofilm formation and the improvement of water quality. Furthermore, the potential functional properties (related to the formation of biofilm and degradation of organic matter) of bacterial community in the V-GO and P-GO biofilms were predicted by the PICRUSt, based on the KEGG database (Figure 6e). The genes related to quorum sensing (QS) in the P-GO biofilm accounted for 1.34%, which was greater than that of the V-GO biofilm (1.18%). Previous studies had shown that QS can promote the aggregation of bacteria or algae [69]. The high proportion of QS in the P-GO biofilm could form a denser biofilm with high hydraulic resistance due to the enhanced bacterial aggregation. In addition, the function associated with biofilm formation, amino acid and polysaccharide biosynthesis displayed a greater proportion in the V-GO biofilm, while the function related to the organic matters degradation in P-GO was richer than that in the V-GO biofilm. These results were consistent with the analysis of microbial community at the genus level.

The analysis of microbial community reveals underlying mechanisms for the formation of biofilms with different properties on the V-GO and P-GO membrane at the microscale. Some EPS secreting bacteria and the genes related to the formation of biofilm accounted for a higher

proportion in V-GO biofilm, suggesting that V-GO membrane cannot effectively mitigate biofilm formation compared to P-GO membrane. For P-GO membrane, the greater abundance of bacteria associated with EPS consumption indicates that P-GO membrane is capable of alleviating biofilm formation. Nevertheless, preventing biofilm formation does not necessarily reduce biofouling because the greater genes related to the QS in P-GO biofilm cause the aggregation of microorganisms and a dense biofilm with directly increased hydraulic resistance. In addition, the movement and predation of microorganism may also play an important role in the structure formation of biofilm, but it requires further investigation. It is worth noting that although the P-GO biofilm had more bacteria with the ability to degrade organic matter, the effluent quality of the P-GO membrane was comparable with the V-GO. This can be ascribed to the following: (i) the abundance of these bacteria in the biofilm was too low to be significant; (ii) the development of the biofilm was not substantial enough due to the insufficient operation period. Thus, taken together, the physical interception rather than biodegradation of the biofilm dominated the whole separation process.

Figure 6

Conclusion and implication

In summary, two representative GO membranes with different surface microstructures (V-GO and P-GO), and a natural surface water, were used to investigate the long-term filtration performance and biofilm characteristics by a GDM filtration system. The results showed that the GO membranes can achieve sustainable water purification over a long-term operation of 45 days, associated with a complete rejection of biopolymers and favorable removal for fluorescent protein-like organic matters. The different microstructure of the V-GO and P-GO membranes surface affected the microorganism colonization and community, resulting in the formation of different biofilms, and in turn determined the permeate flux. The V-GO membrane with a rougher surface, reduced hydrophilicity and higher sheet-edge exposure than that of the P-GO membrane formed a highly heterogeneous and porous biofilm with high permeability,

despite its greater biofilm thickness and EPS content. In contrast, the P-GO biofilm exhibited a thin but dense structure and the hydraulic resistance was approximately 1.5 times higher than that of the V-GO membrane. Furthermore, some EPS producing bacteria was more abundant in V-GO biofilm, indicating that V-GO membrane fails to mitigate biofilm formation. However, bacteria and genes related to the degradation of organic matters and the aggregation of microorganisms accounted for a greater proportion in P-GO biofilm, suggesting that P-GO membrane is capable of alleviating biofilm formation, but forming a dense biofilm with higher hydraulic resistance. To the best of our knowledge, this is the first attempt to evaluate the long-term performance (over 45 days) of GO membranes in practical water environment. The results drawn from the present study highlight the sustainable purification performance of a GO membrane, and provide supporting information for the practical application of GO membranes in water treatment. More importantly, the investigation of biofilm characteristics formed on the GO membranes is particularly important as it has identified the interaction between the GO layer and microorganisms, which are influential in determining the long-term filtration performance and in the design of the GO membrane for specific environmental applications. Further study is required to investigate the impact of filtration mode and operating parameters on the permeation, separation performance and biofouling behavior of GO membranes, in order to further improve the performance and progress the practical application of GO membrane.

Appendix A. Supporting Information

Supporting Information contains twelve figures and one table that complement the results summarized in the paper. These are: the quality parameters of JM water (**Table S1**); schematic diagram of the filtration systems (**Figure S1**); XRD pattern of GO membranes (**Figure S2**); variation of normalized flux of V-GO and P-GO membranes (**Figure S3**); the relationship of flux and biofilm resistance (**Figure S4**); EEM spectra and PARAFAC analysis (**Figures S5-7**); variation of UV_{254} , SUVA, E_{254}/E_{365} and TOC values of the feed and filtrate waters (**Figure S8**); FTIR spectra of the pristine and fouled membranes (**Figure S9**); XPS spectra of V-GO and P-GO biofilms (**Figure S10**); coverage index on OTU level for V-GO and P-GO biofilms

(**Figure S11**); relative abundance of the microbial community composition at phylum level for V-GO and P-GO biofilms (**Figure S12**).

Author contributions

Long Tian and Wenzheng Yu designed the experiments; Long Tian completed the main experiments and prepared the draft manuscript; Peng Zhou was involved in some of the experiments and data analysis; Nigel Graham, Guibai Li and Wenzheng Yu helped to discuss the results and revise the manuscript; Nigel Graham and Wenzheng Yu made the final revision to the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that may have influenced the work reported in this paper.

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Figure Captions

Figure 1. Characterizations of V-GO and P-GO membranes: (a) Top-view SEM images; (b) AFM images and the corresponding roughness, the insets are the analysis of the wrinkles height; (c) temporal variation of the water contact angles during initial 180 s, the insets are the initial contact angle images; (d) FTIR spectra of V-GO and P-GO membranes.

Figure 2. (a) Permeate flux profiles of the V-GO and P-GO filtration systems during the operation period, the inset shows the flux at the 25-46 days. The significance *** represents the $P < 0.001$; (b) change of the biofilm resistance over the operation period, the inset shows the resistance at the 25-46 days; (c) the average filtration resistance distribution at the 25-46 days, R_b and R_m represent the biofilm resistance and the intrinsic membrane resistance, respectively; (d) EEM fluorescence spectra of the raw water, V-GO and P-GO filtrates at the end of operation; (e) molecular weight distributions of organic matters in the feed and filtrates at the end of operation.

Figure 3. SEM and CLSM images of biofilms over 46 days growth on the GO membrane surface: (a-b) are the top view SEM images of (a) V-GO and (b) P-GO biofilms, the inset in (b) represents the enlarged area of the red framed region; (c-d) are the cross-section view SEM images of (c) V-GO and (d) P-GO biofilms; (e-l) are the CLSM images of biofilm formed on the (e-h) V-GO and (i-l) P-GO membrane surface (scale bar, 10 μ m). Blue, green and red represent the eDNA (stained by DAPI), proteins (stained by FITC) and polysaccharides (stained by ConA). (e-g) and (i-k) show the single images, whereas (h) and (l) are the mixed

images of the (e-g) and (i-k), respectively.

Figure 4. Composition and physicochemical properties of biofilms: (a) content of proteins and polysaccharides extracted from the biofilms; (b) molecular weight distribution of organic matters extracted from the biofilms; (c) water contact angle of the biofilms as a function of time, with insets showing the images of contact angle at 100 s; (d) functional group properties of the biofilms as illustrated by ATR-FTIR.

Figure 5. High resolution XPS spectra of biofilm: (a-c) are the C1s, O1s and N1s of the V-GO biofilm; (d-f) are the C1s, O1s and N1s of the P-GO biofilm.

Figure 6. Analysis of microbial community based on high-throughput sequencing: (a) Venn diagram on OTU level; (b) Chao index and Shannon index on OTU level; (c) Relative abundance of the microbial community composition at class level; (d) Relative abundance of the microbial community composition at genus level; (e) PICRUSt function prediction of the microbial community based on KEGG database at level 3.

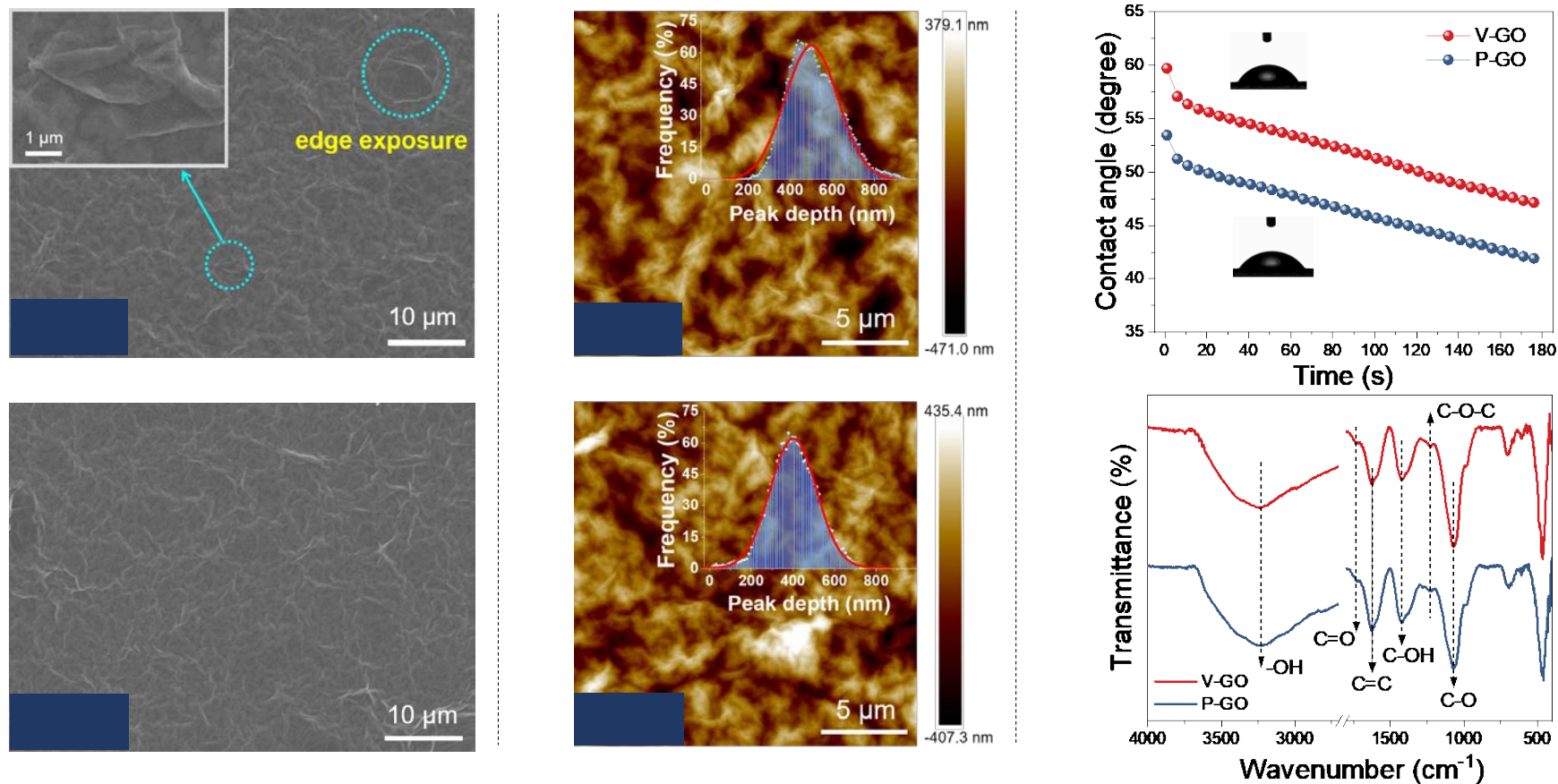


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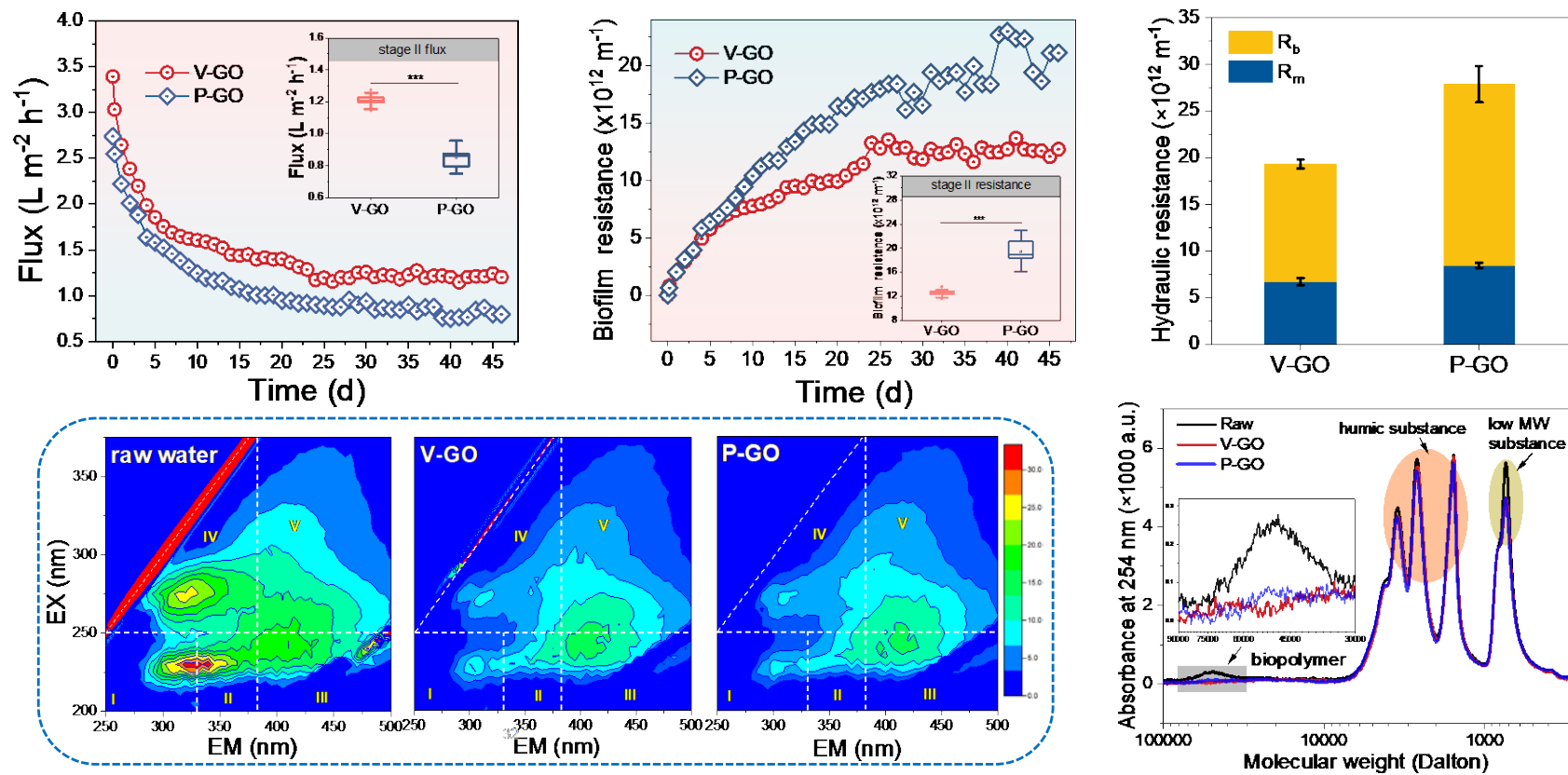


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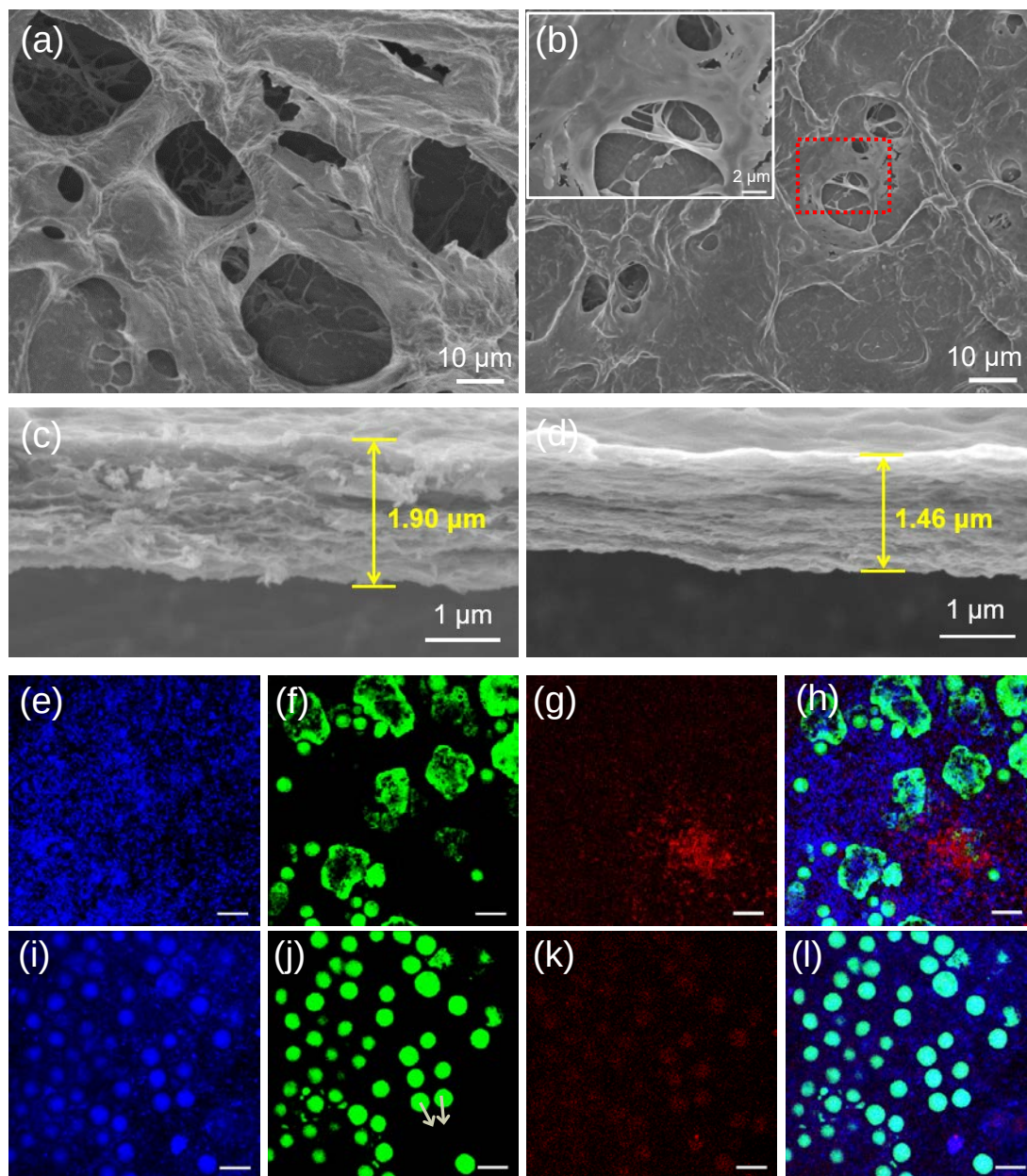


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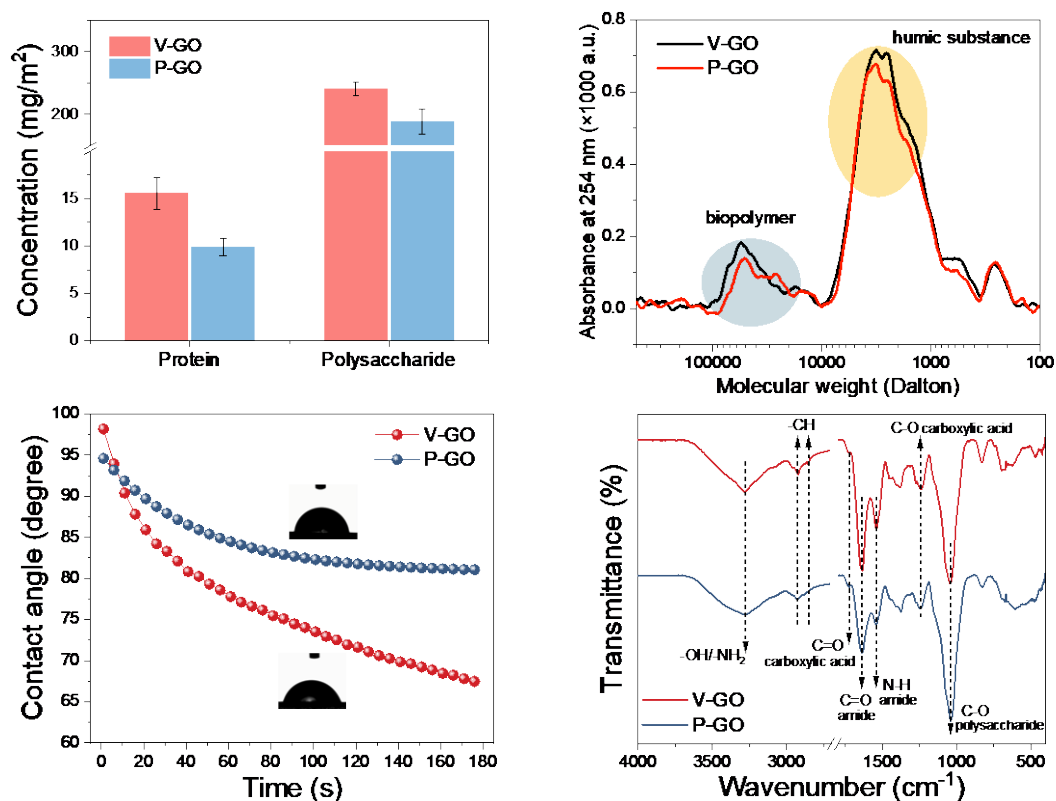


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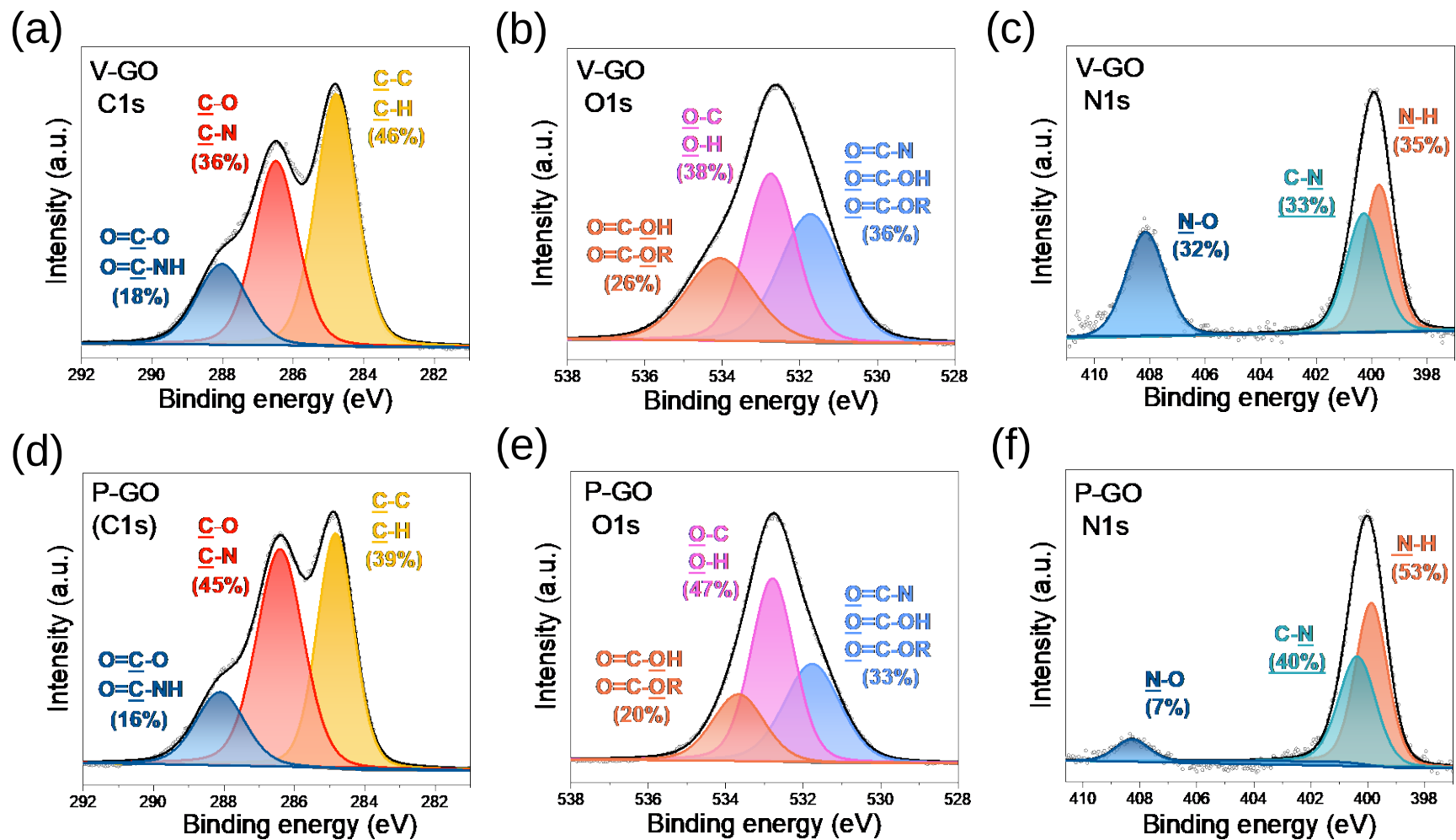


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