# Surface-independent one-pot chelating copper (II) ions on filtration membranes for antibacterial properties

Zhongyun Liu[1], Yunxia Hu[1*], Caifeng Liu[1,3], Zongyao Zhou[1,2]

1. CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation；

Research Center for Coastal Environmental Engineering and Technology of Shandong Province；

Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences,

Yantai, Shandong Province 264003, P.R. China

2. University of Chinese Academy of Sciences, Beijing 100049, P.R. China;

3. College of Chemistry and Chemical Engineering, Yantai University, Yantai, Shandong Province 264000, P.R. China

*Corresponding author, Tel: +86-535-2109236; E-mail: yunxiahu@yic.ac.cn

## Experimental Details

**Materials:** Dopamine (DOPA) hydrochloride and Polysulfone (Psf, Mn: 22 000 Da) were purchased from Sigma Aldrich (St. Louis, MO, USA). 1 M Tris-HCl buffer (pH 9.0) was purchased from Beijing Solarbio Science & Technology Co., Ltd, China. Copper (II) sulfate pentahydrate, hydrochloric acid and other chemical agents were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd, China and used as received. SYTO®9 green fluorescent nucleic acid stain was purchased from Invitrogen (Eugene, Oregon, USA).

Polysulfone and Polyvinylidene Fluoride (PVDF, 6010, Solvay) ultrafiltration (UF) membrane were fabricated by non-solvent induced phase separation, adapting the procedure in previous reported methods.[1-2] The commercial thin film composite forward osmosis (TFC FO) membranes were purchased from Hydration Technology Innovations (HTI, Albany, OR, USA), and the membrane coupons wetted in 25% isopropanol solution for 30 min, followed by thorough rinse with deionized (DI) water and stored at 4 °C before further modification.

**Surface Modification of Filtration Membranes**

74.94 mg of copper (II) sulfate ($CuSO_4$) pentahydrate power (3 mmol) was dissolved in 100 mL of Tris-HCl buffer solution (50 mM, pH 8.5), further adjusting its pH to 4.5 with 1 M HCl to prepare $CuSO_4$ solution (30 mM). The pH value of the $CuSO_4$ solution was measured with a pH meter (PB-10, Sartorius, Germany). Then the DOPA-$Cu^{2+}$ solution was prepared through the addition of 200 mg of DOPA to the fresh prepared $CuSO_4$ solution. The concentration of DOPA in the prepared DOPA-$Cu^{2+}$ solution was 2 mg/mL.

The filtration membrane coupons were soaked in the fresh prepared DOPA-$Cu^{2+}$ solution in a glass pan open to air and kept agitated in shaking incubator (THZ-82A, Kexi Instrument, China)

for 1 h at room temperature. Then, the membrane coupons were thoroughly rinsed with deionized water three times to obtain the polydopamine coated filtration membrane, and dried at ambient conditions. As controls, 2 mg/mL DOPA Tris-HCl buffer solutions (50 mM, pH 8.5 and 50 mM, pH 4.5, without copper ions) were also prepared and surface modification of filtration membranes was also carried out in different conditions listed in Table S1with the aforementioned processes.

For surface modification of Psf UF membranes without air involved, 100 mL of fresh prepared $CuSO_4$ solution (30 mM $CuSO_4$, 50 mM Tris-HCl, pH 4.5) was added into a two-neck round bottom flask equipped with a magnetic stirrer. Three cycles of freeze-pump-thaw were carried out to remove the oxygen in the $CuSO_4$ solution. Upon purging with nitrogen, 100 mg of DOPA was added into the solution. After DOPA being dissolved, Psf UF membrane coupons were placed into the above DOPA-$Cu^{2+}$ solution, followed by another three cycles of freeze-pump-thaw to make the above reaction solution under deoxygenated condition. After 1-hour incubation of Psf UF membranes in the above DOPA-$Cu^{2+}$ solution, Psf UF membranes were taken out rapidly and thoroughly rinsed with deionized water three times, and dried at ambient conditions.

**Table S1.** Different modification conditions of Psf UF membranes

| Membranes | Concentration of DOPA | Copper (II) ions | Air | Tris-HCl buffer | pH |
|---|---|---|---|---|---|
| **a** | ----- | ----- | ----- | ----- | ----- |
| **b** | 2 mg/mL | ----- | √ | 50 mM | 8.5 |
| **c** | 2 mg/mL | ----- | √ | 50 mM | 4.5 |
| **d** | 2 mg/mL | 30 mM | √ | 50 mM | 4.5 |
| **e** | 2 mg/mL | 30 mM | ----- | 50 mM | 4.5 |

**Membrane Characterization**

The morphologies of the pristine and modified membrane surfaces were observed by scanning electron microscopy (SEM, S-4800, Hitachi, Japan). All samples were dried in a vacuum oven for 12 hours at 50 °C, and then were coated with 10 nm thick platinum (Pt) for 100 seconds using an EMITECH SC7620 sputter coater before SEM observation. Existence and distribution of copper ions in the membranes were confirmed by EX-350 Energy Dispersive X-ray Microanalyzer (EDX, Horiba, Tokyo, Japan). The static contact angles of modified membranes were detected using an optical instrument (ADS300, Data Physics, Germany). Using the sessile drop method, a 2 μL droplet was placed on the air-dried membrane surfaces for 5 seconds, and photographed with a digital camera. To account for variations in the measurements, we performed ten measurements on each sample and tested three individual samples for parallel experiments. The elemental composition of the membrane surfaces and the forms of the chelated copper ions were also detected using X-ray photoelectron spectroscopy and (XPS, Thermo Escalab 250Xi, Thermo Fisher Scientific, USA) with monochromatic Al-Ka X-ray source (hv=1486.6 eV). All binding energies (BEs) were referenced to that of the neutral C 1s hydrocarbon peak at 284.6 eV. The photoelectron take-off angle was 60 ° and the full range data was collected by survey scan (200–1200 eV, step size: 1 eV). 1 mL of DOPA solutions with different reaction conditions at different time (0 min, 10 min, 20 min, 30 min, 40 min) was taken and diluted to 3 mL to measure the UV-vis absorbance at 420 nm with an UV-Vis spectrometer (TU-1810, Persee, China).[3]

**Antibacterial Activities of Modified Filtration Membranes**

Gram-positive *Staphylococcus aureus* (*S. aureus*) and the colony forming unit (CFU) counting

method[4-5] were used for the evaluation of the antibacterial activities of the modified membranes. An overnight culture of bacteria (1 mL) in Luria-Bertani (LB) broth was diluted in 20 mL fresh LB broth and then grew for 4-5 h to reach the mid-exponential growth phase. The bacteria culture was centrifuged for 1 min at 5000 rpm to remove the supernatant, washed with PBS twice, and then re-suspended with physiological saline solution (0.15 M NaCl, pH 7.0, 20 mM $NaHCO_3$) to initial optical cell densities at 600 nm ($OD_{600}$) of 0.15 ± 0.09. Circular membrane coupons with 1.6 cm in diameter were placed in sterile plastic tubes with 10 mL of the bacteria in physiological saline solution (0.15 M NaCl, pH 7.0, 20 mM $NaHCO_3$). After incubation for 5 h at 37 °C, the membranes were rinsed gently three times with DI water to remove unattached bacteria. Membrane coupons were then sonicated in a bath sonicator (KQ5200DE, Kun Shan Ultrasonic Instruments Co., Ltd, China) for 7 min at 25 °C in 10 mL of physiological saline solution to remove the attached bacteria. The suspension was serially diluted 100 times, and 100 μL of the bacterial solution was taken to plate on LB agar plates. Then bacteria colonies were counted after overnight incubation. The antibacterial efficiency ($E_b$) was measured from Eq. (1).

$$E_b = \left(\frac{N_p - N_m}{N_p}\right) \times 100\% \quad (1)$$

Where $N_p$ and $N_m$ are the numbers of colonies corresponding to the pristine membranes and the modified membranes, respectively.

The live bacteria attached on the membrane coupons were also determined using confocal laser scanning microscopy (Fluo View FV1000, Olympus, Japan). For fluorescence imaging, circular membrane coupons were incubated for 24 h at 37 °C with the aforementioned approaches, and were rinsed gently three times with DI water to remove unattached bacteria. Then circular membrane coupons were placed into a 12-well tissue culture plate and covered with 1 mL of 3.34

μM SYTO@9 solution for 15 min at 37 °C.[6] After that, the membranes were rinsed twice with DI water and imaged with confocal laser scanning microscopy (excitation with an argon laser at 488 nm, emission at 503 nm).

**Membrane Filtration Performance**

The water fluxes of pristine and modified Psf UF membrane and PVDF UF membrane were measured with a dead-end filtration system. In a typical procedure, the membranes were mounted on the membrane cell with an effective area of 3.85 $cm^2$ and pre-compacted with deionized water at 1.5 bar for 30 min. Then the pure water flux was measured by recording the volume of the permeated water at a transmembrane pressure (TMP) of 1.0 bar and an ambient temperature of 25 ± 1 °C. The pure water flux ($J_w$)was calculated by Eq. (2).

$$J_w = \frac{V}{t \times S} \tag{2}$$

where V (L) was the volume of the permeated pure water, S ($m^2$) was the effective membrane area and t (h) was the recorded time.

The BSA rejection of pristine and modified Psf UF membrane was determined by replacing deionized water with 1.0 g/L of BSA solution (PBS, pH 7.4). With the same pre-compacted process, the permeated solution was collected at a TMP of 1.0 bar and an ambient temperature of 25 ± 1 °C, and the BSA rejection (R) was calculated by Eq. (3).

$$R = \left(1 - \frac{C_{BSA-P}}{C_{BSA-F}}\right) \times 100\% \tag{3}$$

where $C_{BSA-P}$ and $C_{BSA-F}$ were the concentration of BSA in the permeate and the feed solutions, respectively, which were measured by UV-vis spectrometer at 280 nm.

The water flux and the reverse salt flux of TFC FO membrane before and after modification were

measured with a FO test system. The effective surface area of the membrane was 38.52 $cm^2$. The draw solution and feed solution were 1 M NaCl solution and deionized water, respectively. The FO test ran at 25 ºC ± 0.1 ºC with 0.5 L/min of both cross flow velocities of feed and draw solution. The membranes were evaluated under AL-DS (active layer facing draw solution) operational mode. The water flux was calculated as the water weight gain of the draw side per hour and per area of the membrane, and the salt flux was calculated as the salt weight gain of the feed side per hour and per area of the membrane. The water weight gain was monitored by the balance (ME3002, Mettler-Toledo, Switzerland), and the salt weight gain was monitored by the conductivity meter (CON2700, Eutech, USA).

**Evaluation of Copper Ions Release**

To test the contents of Cu ions in the filtered water leaking from the membranes, the experiments were performed to determine the level of Cu ions in the permeate filtered through the Cu-containing Psf UF membrane with an effective membrane area of 3.85 $cm^2$ in a dead-end filtration system under the pressure of 1.0 bar at an ambient temperature 25 ± 1 °C. At predetermined time intervals, 15 mL of the permeate after filtered through the membranes was taken out and acidified with 0.5 mL of 68% nitric acid for ICP-MS measurements (Inductively Coupled Plasma Mass Spectrometry, ELAN DRC II, PerkinElmer (Hong Kong) Ltd.).

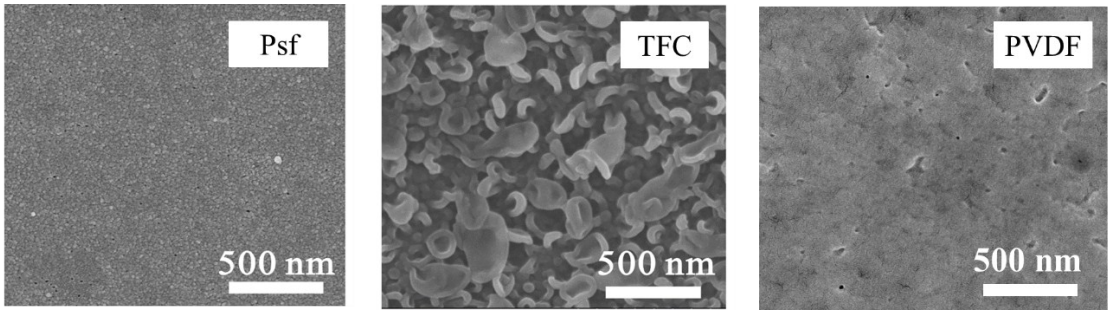


**Figure S1.** SEM micrographs of the pristine Psf UF membrane, TFC FO polyamide membrane and PVDF UF membrane.

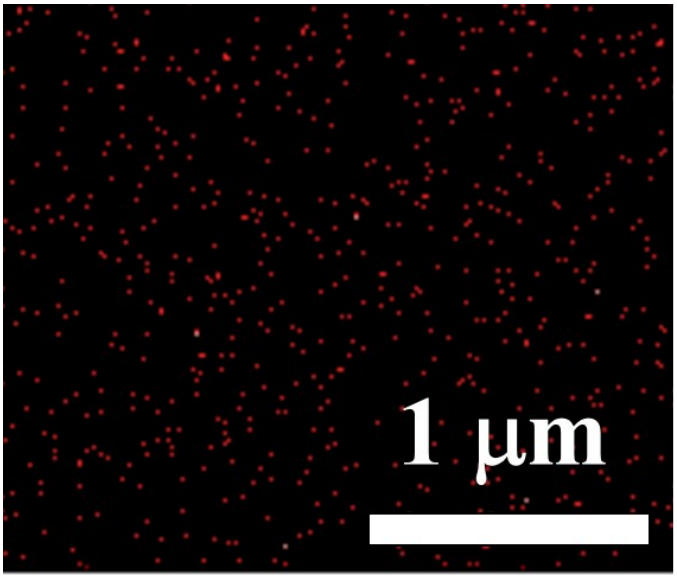


**Figure S2.** The distribution of copper (Cu) element on the modified Psf UF membrane surface, and copper was observed as red points by EDX mapping analysis

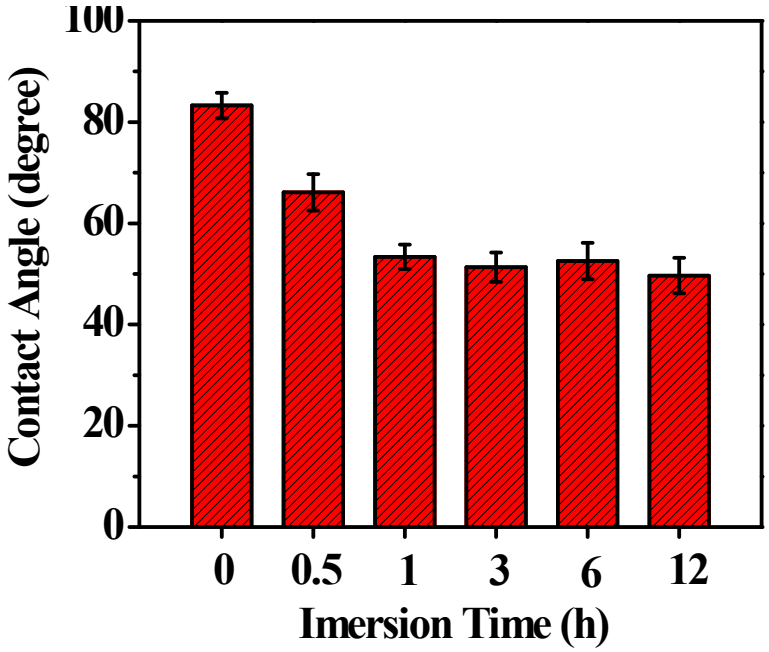


**Figure S3.** The water contact angels of Psf UF membranes after soaking in 2 mg/mL dopamine solution containing 30 mM $CuSO_4$ at 50 mM Tris-HCl buffer (pH 4.5) for a period of time varying from 0, 0.5 h, 1 h, 3 h, 6 h, and to 12 h.

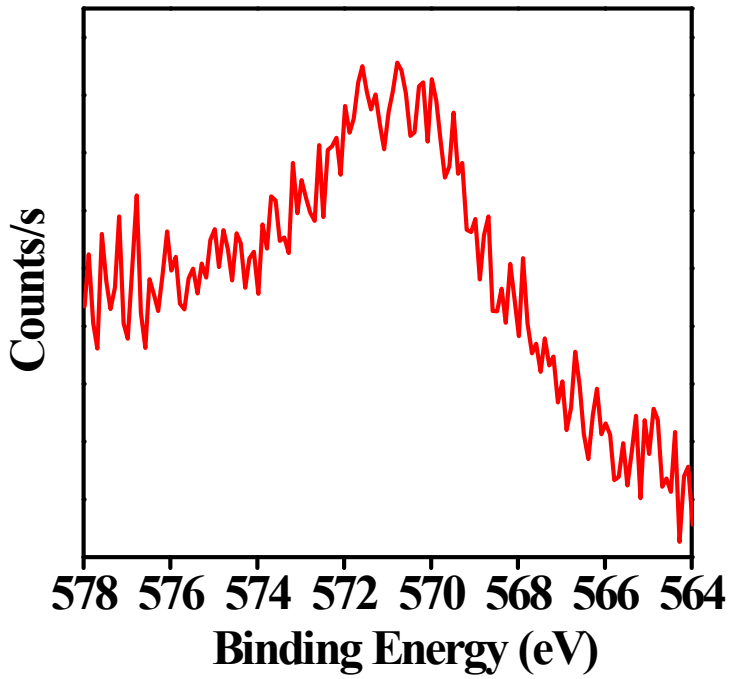


**Figure S4.** Cu LMM XP spectra of copper chelated Psf UF membrane surface.

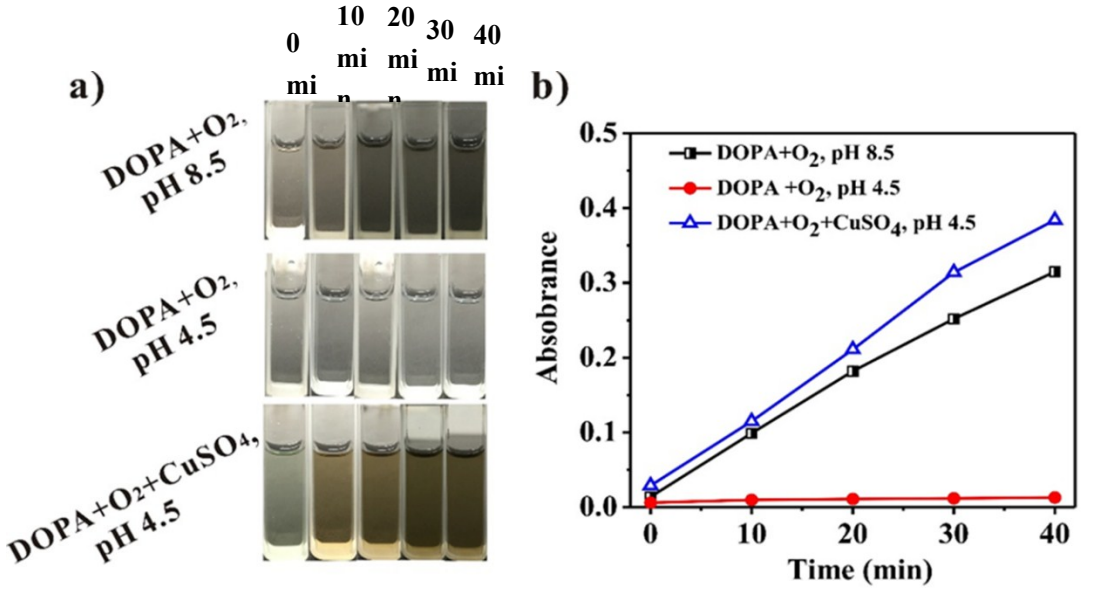


**Figure S5.** a) Photographs of dopamine solutions in air at various reaction conditions for a certain time period from 0 min, 10 min, 20 min, 30 min, to 40 min; b) Time-dependent absorbance of DOPA solution at 420 nm at various conditions.

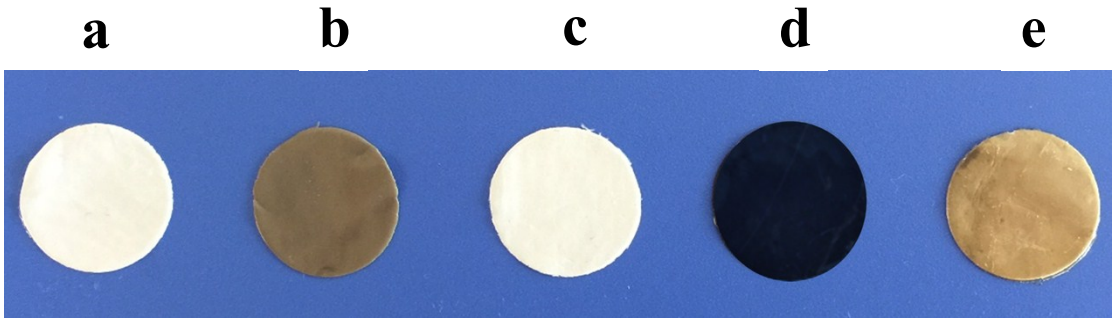


**Figure S6.** Photographs of Psf UF membrane coupons upon 1 hour immersion in different DOPA solutions: a) pristine membrane; b) 2 mg/mL DOPA solution in 50 mM Tris-HCl buffer at alkaline conditions (pH 8.5); c) 2 mg/mL DOPA solution in 50 mM Tris-HCl buffer at acidic conditions (pH 4.5); d) 2 mg/mL DOPA solution containing 30 mM $CuSO_4$ in 50 mM Tris-HCl buffer at acidic conditions (pH 4.5); e) 2 mg/mL DOPA solution with 30 mM $CuSO_4$ in 50 mM Tris-HCl buffer at acidic conditions (pH 4.5) but in deoxygenated condition.

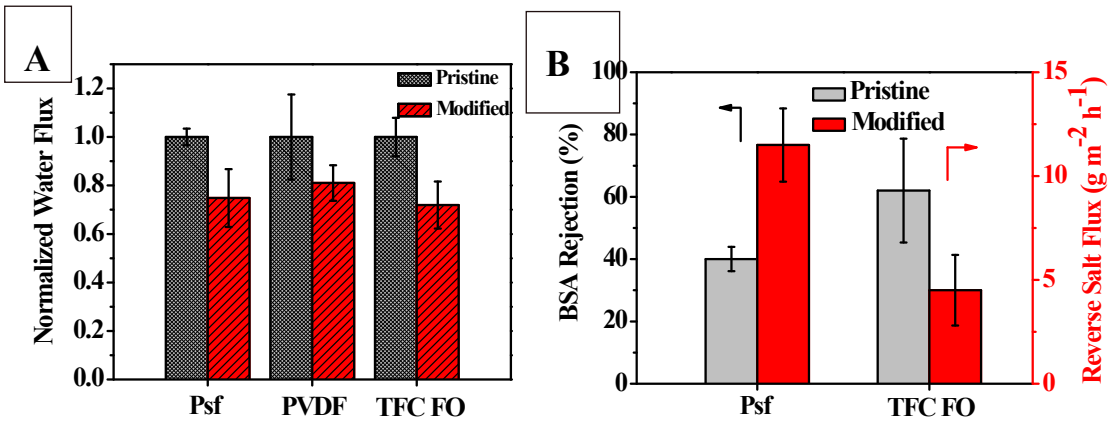


**Figure S7.** Normalized water flux of pristine and modified Psf UF membrane, PVDF UF membrane and TFC FO membrane (A), and separation efficiency of pristine and modified Psf UF membrane and TFC FO membrane, respectively (B).

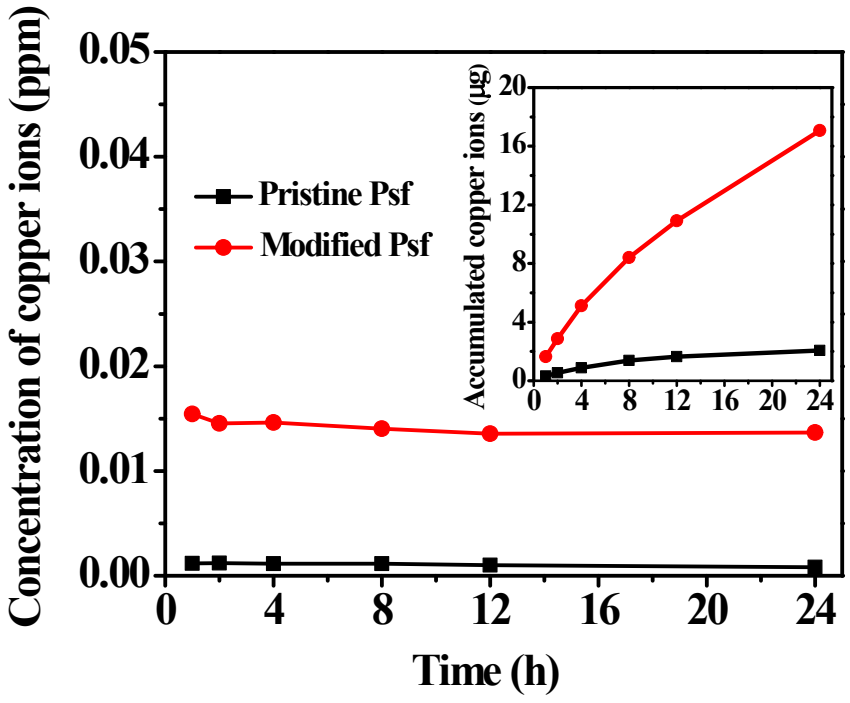


**Figure S8.** Concentration profile of copper ions and the accumulated copper ions (insert) in the permeates with different filtration time.

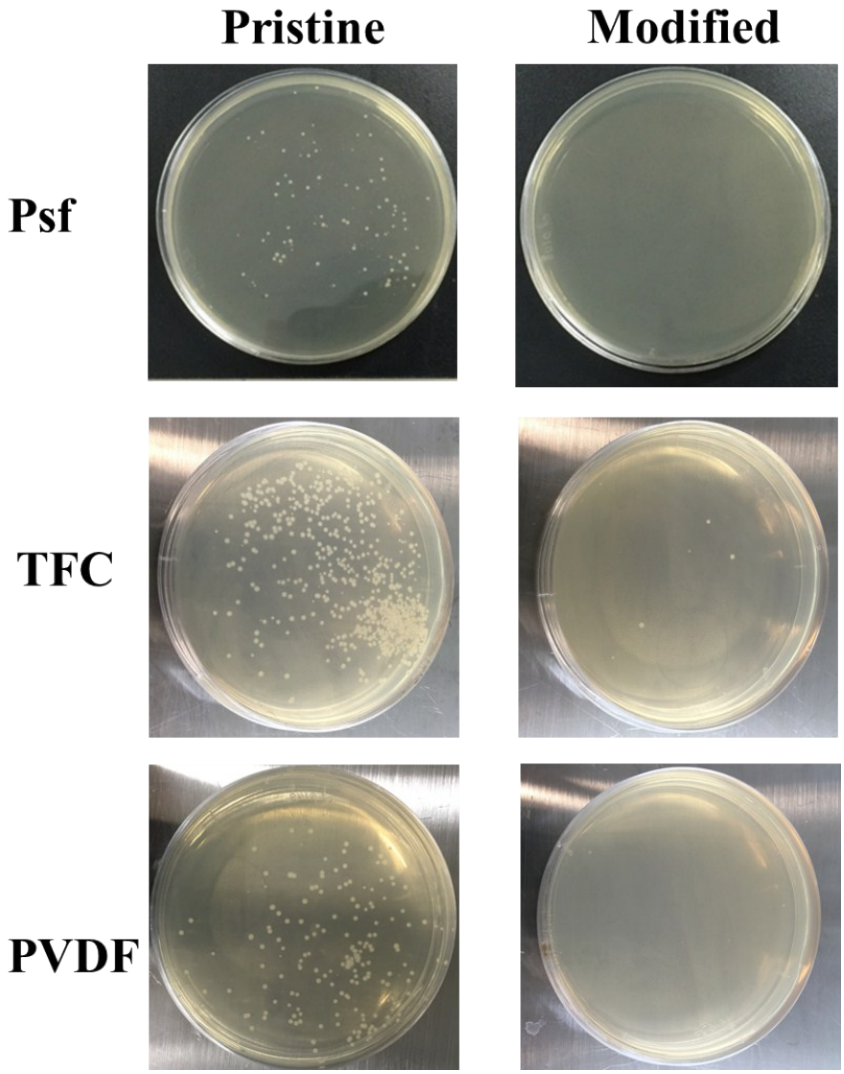


**Figure S9.** The representative bacterial culture plate photographs of samples from the pristine and modified Psf UF membranes, TFC FO membranes and PVDF UF membranes before and after chelating copper ions.

**References**

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