

Coupling Cu₂O Clusters and Imine-linked COFs on Microfiltration Membranes for Fast and Robust Water Sterilization

Corresponding Author: Professor Yong Wang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

In this manuscript, the authors have reported the synthesis of Cu₂O Clusters and Imine-linked COFs on Microfiltration Membranes and used them against both *E. coli* and *S. aureus*. However, the manuscript needs some essential revisions which would enhance the quality of the manuscript if included. Therefore, this work only can be considered for after the following major revisions:

1. A table comprising of various copper based based antibacterial activities should be compared to the present work and must be included in the manuscript.
2. Authors are encouraged to discuss about other methods of metal chalcogenide synthesis like electrodeposition, chemical bath deposition, sintering, sol-gel synthesis method. Authors are encouraged to discuss briefly about other Cu and silver based conventional antibacterial agents and how the present work is more efficient than other published work. Agents Chemother. 2015, 59(11), 6882; Langmuir 2019, 35, 52, 17166.
3. Authors need to refine their English and correct the multiple grammatical errors throughout the manuscript.
4. Authors are advised to provide a schematic representation showing the damaging effect of the Cu₂O cluster based membrane on the bacterial cells.
5. Authors are encouraged to write sentences in active voice. For eg . the following sentence can be corrected to "In multiple fields including healthcare services^{1, 2}, food processing³, and 29 livestock farming⁴, the elimination of bacterial contamination in water has 30 become a core safety indicator." "Elimination of bacterial contamination in water has become a core safety indicator in many fields like healthcare services^{1, 2}, food processing³, and 29 livestock farming⁴ etc.

Reviewer #2

(Remarks to the Author)

In this manuscript, the authors designed a bactericidal microfiltration membrane through sequential deposition of an imine-linked COF (TbPa) layer and nanosized Cu₂O clusters. This membrane integrates excellent permeation flux, high bacterial killing efficiency and effective biofilm removal. Moreover, the authors elaborately revealed the antifouling and bactericidal mechanisms by studying the photoelectric performance, ROS generation and bacteria inactivation of this membrane. This interesting study demonstrated a new approach for the water sterilization using photocatalytic microfiltration membranes, and thus may inspire more researchers to investigate other photoelectric materials in this application scope. I recommend this manuscript for publication in Nature Communications after addressing the following issues:

1. Line 19-22 on Page 2: '...', thereby enhancing the performance of the membrane in generating reactive oxygen species (ROS). Consequently, this membrane demonstrates exceptional water flux performance, ...'. The word 'Consequently' is confusing, why can the enhanced ROS generation cause exceptional water flux performance?
2. Figure 1 on Page 6: (1) The colors of bacteria and Cu₂O clusters are similar. It would be better if the color could be optimized. (2) The arrows are confusing. What causes the bactericidal effect and biofilm removal? (3) The color for the light irradiation is somewhat hard to distinguish; it could be made more prominent for better visibility. (4) The 'sustained operational stability' mentioned in the caption is not reflected in the schematic diagram.

3. Figure 2 on Page 7: (1) What do the small black spheres in Figure 2 represent? (2) What effect does high temperature (220°C) have on the PES membrane? (3) If Figures 2 and 3 could be combined and simplified, it might provide readers with a clearer and more intuitive understanding of the membrane.
4. Line 124-125 on Page 8, '... a minimal amount of copper is incorporated onto TbPa'. Is there any quantitative data available?
5. Figure 3g on Page 10, what is the morphology of TbPa COFs in the SEM image?
6. Line 185 and 187 on Page 11, why do Figure 3j and 3k demonstrate the regular and ordered anchoring of Cu₂O clusters? Do they show a periodic arrangement?
7. Line 211-212 on Page 12, what does the band energy of TbPa (~ 2.52 eV) mean?
8. What is the 'LZU1' in Figure 5e, 5f, and 5h?
9. Figure 7e on Page 28, why don't the dead bacteria clog the membrane? After bacterial lysis, could toxic intracellular contents leak into the filtrate?
10. Line 515-516 on Page 28, 'it excels in membrane separation performance'. This sentence is confusing, what substances can be separated using this membrane? Are there any experimental data available?
11. Can this membrane kill harmful fungi?

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have made appropriate corrections to the comments of manuscript. The modified and added contents are highlighted. This manuscript is suitable for publication in the present form.

Reviewer #2

(Remarks to the Author)

the authors have properly revised the manuscript

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Responses to comments

Reviewer #1

In this manuscript, the authors have reported the synthesis of Cu₂O Clusters and Imine-linked COFs on Microfiltration Membranes and used them against both E. coli and S. aureus. However, the manuscript needs some essential revisions which would enhance the quality of the manuscript if included. Therefore, this work only can be considered for after the following major revisions:

Comment 1. A table comprising of various copper based antibacterial activities should be compared to the present work and must be included in the manuscript.

Response: We followed this insightful suggestion and provided such a table in revision as Supplementary Table 2:

Supplementary Table 2. Summary of the synthetic methods, antibacterial efficacy and mechanism of Cu-based materials

Material	Synthetic method	Antibacterial efficacy	Mechanism	Ref
Cu ₂ O nanoparticles	solution synthesis	625 µg/ml MIC ⁸ of <i>S. aureus</i>	sustained release of Cu ²⁺	S1
Cu ₂ O nanopetals@ Cu foil	electrodeposition	87.96% of <i>E. coli</i>	sustained release of Cu ⁺	S2
BSA ¹ -CuS nanoparticles	solution synthesis	80% of <i>S. aureus</i> and <i>E. coli</i> under NIR ⁹	photothermal therapy	S3
MoS ₂ @ copper peroxide nanodots	solution synthesis	inactivated 3 log of <i>E. coli</i> with H ₂ O ₂ under NIR	photothermal/photodynamic therapy and enzyme-like catalysis therapy	S4
Bio-CuSe	solution synthesis	>95% of <i>E. coli</i> under NIR light.	photothermal therapy	S5
<i>E. coli</i> -antibiotic orthogonal Cu-based material	solution synthesis and sintering	~80% <i>E. coli</i> with precursor molecules	bio-orthogonal catalysis therapy	S6
PdCu-Urchin	solution synthesis	~92% <i>E. coli</i> with H ₂ O ₂	enzyme-like catalysis therapy and bio-orthogonal catalysis therapy	S7
Cu nanowires-on-PMMA ²	solvothermal synthesis	90% <i>E. coli</i>	sustained release of Cu ²⁺	S8
Cu nanoparticle-coated cellulose film	solution synthesis	3-log reduction of <i>E. coli</i>	sustained release of Cu ²⁺	S9
Cu ion-modified PVF ³	solution synthesis	~96% of <i>S. aureus</i>	sustained release of Cu ²⁺	S10
Cu _x S	hot injection	74.48 % of <i>E. coli</i> under NIR	photothermal therapy	S11
CuO-HCSs ⁴	chemical bath deposition	inactivated 2.6 log of <i>E. coli</i> with H ₂ O ₂	enzyme-like catalysis therapy	S12
Cu-MOF-1/PLA ⁵ fibrous membrane	microwave synthesis	99.3% of <i>E. coli</i>	sustained release of Cu ²⁺	S13
copper clusters	solution synthesis	73.11% of <i>E. coli</i>	sustained release of Cu ²⁺	S14
0.5Cu coating	sol-gel synthesis	~98% of <i>E. coli</i>	sustained release of Cu ²⁺	S15
MPG ⁶ -Cu ₅	sol-gel synthesis	inactivated 3 log of <i>E. coli</i>	sustained release of Cu ²⁺	S16
Cu-PBG ⁷	electrodeposition	88.3 % of <i>E. coli</i> with H ₂ O ₂	nanozymes and sustained release of Cu ²⁺	S17
Cu ₂ O/TbPa/PES	vapor deposition and photodeposition	5-log reduction of <i>E. coli</i> and <i>S. aureus</i> under visible light	photocatalysis	This work

Note (s):

¹BSA, Bovine Serum Albumin.

²PMMA, Polymethyl Methacrylate.

³PVF, Polyvinylfluoride.

⁴HCS, Hollow Carbon Spheres.

⁵PLA, Polylactic Acid.

⁶MPG, Mesoporous Phosphate-based Glasses.

⁷PBG, Phosphate-Based Glass.

⁸MIC, Minimum Inhibitory Concentration.

⁹NIR, Near Infrared.

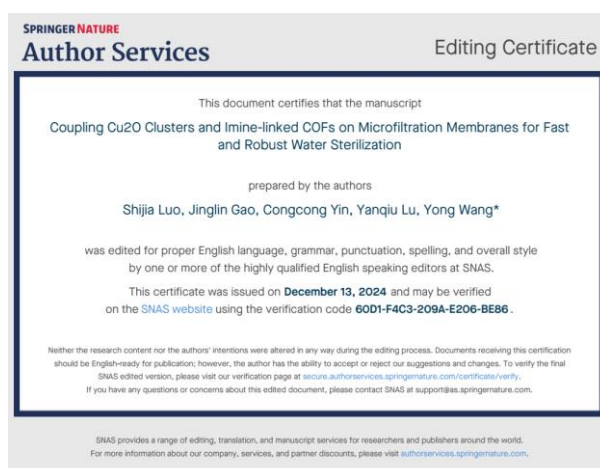
Comment 2. Authors are encouraged to discuss about other methods of metal chalcogenide synthesis like electrodeposition, chemical bath deposition, sintering, sol-gel synthesis method. Authors are encouraged to discuss briefly about other Cu and silver based conventional antibacterial agents and how the present work is more efficient than other published work. *Agents Chemother.* 2015, 59(11), 6882; *Langmuir* 2019, 35, 52, 17166.

Response: We summarized the synthesis method and bactericidal efficiency of Cu-based antibacterial materials reported in the literature in Supplementary Table 2, and added the corresponding discussion to emphasize the better bactericidal performance of our membranes in Page 19 as below:

“In Supplementary Table 2, we carry out a comparative analysis of the synthesis method, bactericidal mechanisms, and antibacterial efficacies of various copper-based materials. Notably, in comparison with other Cu₂O-based materials^{26, 27}, the Cu₂O/TbPa/PES membrane with lower Cu content shows outstanding antibacterial performance by achieving a 5-log reduction against both *E. coli* and *S. aureus* within 30 min.”

Comment 3. Authors need to refine their English and correct the multiple grammatical errors throughout the manuscript.

Response: We have thoroughly proofread the manuscript multiple times and utilized professional editing and revision services provided by Springer Nature Author Services to correct grammatical errors and enhance its readability. The Editing Certificate is provided below:



Comment 4. Authors are advised to provide a schematic representation showing the damaging effect of the Cu₂O cluster based membrane on the bacterial cells.

Response: We followed this very helpful suggestion and incorporated a schematic diagram in Figure 6g to explain how the Cu₂O/LZU1/PES membrane causes bacterial cell death. The added schematic diagram and its description are as follows:

“We have summarized the inactivation mechanism of Cu₂O/TbPa/PES on bacteria into a schematic diagram, as illustrated in Figure 6g.”

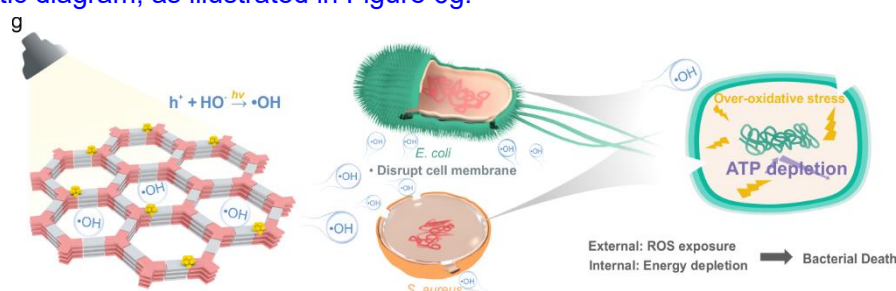


Figure 6. (g) Schematic illustration of the bacterial inactivation mechanism of the Cu₂O/LZU1/PES membrane.

Comment 5. Authors are encouraged to write sentences in active voice. For eg . the following sentence can be corrected to

“In multiple fields including healthcare services^{1, 2}, food processing³, and 29 livestock farming⁴, the elimination of bacterial contamination in water has 30 become a core safety indicator.”

“Elimination of bacterial contamination in water has become a core safety indicator in many fields like healthcare services^{1, 2}, food processing³, and 29 livestock farming⁴ etc.

Response: We have revised the manuscript according to this suggestion, converting passive voice to active voice where applicable. In addition to the sentence mentioned by the reviewer, we have also re-written some sentences in active voice such as:

“Microfiltration membrane technology offers a pivotal solution for effective and sustainable disinfection, featuring high throughput, low energy consumption, and the complete absence of chemical residues.”

“However, compatibility challenges between polymeric substrates and inorganic oxidants cause detachment and wear issues in practical applications.”

Reviewer #2

In this manuscript, the authors designed a bactericidal microfiltration membrane through sequential deposition of an imine-linked COF (TbPa) layer and nanosized Cu₂O clusters. This membrane integrates excellent permeation flux, high bacterial killing efficiency and effective biofilm removal. Moreover, the authors elaborately revealed the antifouling and bactericidal mechanisms by studying the photoelectric performance, ROS generation and bacteria inactivation of this membrane. This interesting study demonstrated a new approach for the water sterilization using photocatalytic microfiltration membranes, and

thus may inspire more researchers to investigate other photoelectric materials in this application scope. I recommend this manuscript for publication in Nature Communications after addressing the following issues:

Comment 1. *Line 19-22 on Page 2: ‘..., thereby enhancing the performance of the membrane in generating reactive oxygen species (ROS). Consequently, this membrane demonstrates exceptional water flux performance, ...’. The word ‘Consequently’ is confusing, why can the enhanced ROS generation cause exceptional water flux performance?*

Response: We are sorry for the confusion. In the original manuscript, the term ‘enhanced ROS generation’ primarily influenced the membrane's antifouling capabilities, encompassing the removal of bacterial fouling and the flux recovery rate. Conversely, the outstanding water flux performance is predominantly ascribed to the membrane's ultrathin deposition and the incorporation of nanoscale Cu₂O clusters. To avoid any confusion, we have undertaken a revision of the manuscript on page 2, as detailed below:

“As bacterial contamination crises escalate, the development of advanced membranes possessing both high flux and antibacterial properties is of paramount significance for enhancing water sterilization efficiency. Herein, an ultrathin layer of TbPa (an imine-linked covalent organic framework) and nanosized Cu₂O clusters, **sequentially deposited onto polyethersulfone membranes, demonstrate exceptional water flux performance, reaching a permeance level of 16000 LHM bar⁻¹.** The deposited TbPa, generating uniformly distributed reduction sites under illumination, facilitates the uniform formation of Cu₂O clusters. Furthermore, these anchored Cu₂O clusters significantly optimize electron transport within the ultra-thin layer of TbPa, thereby enhancing the performance of the membrane in generating reactive oxygen species (ROS). Consequently, **this membrane achieves a flux recovery rate exceeding 98.6% for flux losses caused by bacterial fouling and maintains consistent performance over 10 cycles.** This work presents an effective strategy for accessing bactericidal membranes and provides new insights into efficient and mild water sterilization.”

Comment 2. *Figure 1 on Page 6: (1) The colors of bacteria and Cu₂O clusters are similar. It would be better if the color could be optimized. (2) The arrows are confusing. What causes the bactericidal effect and biofilm removal? (3) The color for the light irradiation is somewhat hard to distinguish; it could be made more prominent for better visibility. (4) The ‘sustained operational stability’ mentioned in the caption is not reflected in the schematic diagram.*

Response: Following these very helpful suggestions, we made the following revisions:

(1) We have adjusted the color of the Cu₂O clusters to yellow in order to distinguish them from bacteria.

(2) The substance responsible for exerting bactericidal activity and biofilm removal is hydroxyl radicals (•OH). To augment the comprehension of the manuscript, we have incorporated several legends depicting •OH adjacent to the arrows.

(3) The color of the light irradiation has been adjusted to orange, and its saturation has been enhanced for prominent display.

(4) We have added an annotation of "Stable operation" in Figure 1 to highlight this important characteristic.

The adjusted Figure 1 is as follows:

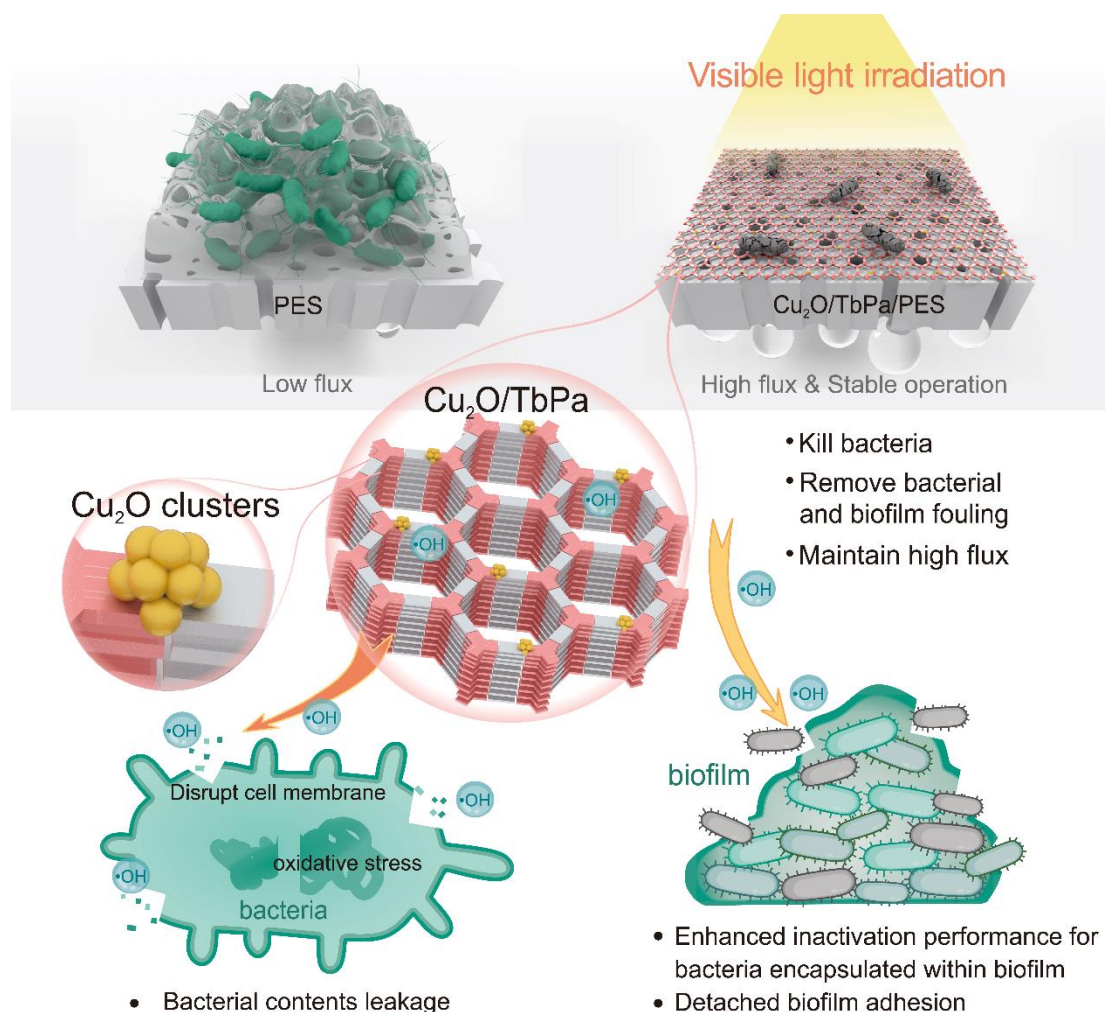


Figure 1. Schematic diagram of $\text{Cu}_2\text{O/TbPa/PES}$ membrane resistance to bacterial fouling. Specifically, the conventional PES membrane experiences a significant decrease in flux due to bacterial fouling. In comparison, $\text{Cu}_2\text{O/TbPa/PES}$, featuring an ultra-thin $\text{Cu}_2\text{O/TbPa}$ layer, effectively inactivates bacteria and disrupts biofilms under visible light irradiation, thereby providing high flux performance, robust anti-fouling characteristics and sustained operational stability.

Comment 3. Figure 2 on Page 7: (1) What do the small black spheres in Figure 2 represent? (2) What effect does high temperature (220°C) have on the PES membrane? (3) If Figures 2 and 3 could be combined and simplified, it might provide readers with a clearer and more intuitive understanding of the membrane.

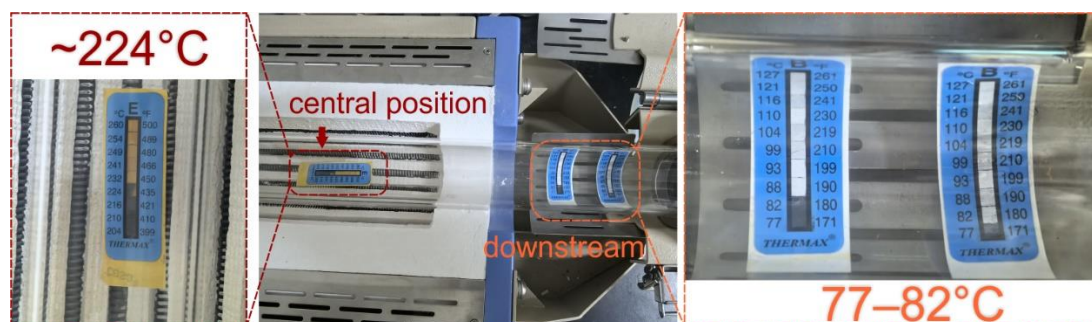
Response:

(1) The small black spheres represent iron powder, which serves to prevent the oxidation of *p*-phenylenediamine (Pa). We have added an annotation in Figure 2 to clarify this point.

(2) The 220°C is the central temperature of the tubular furnace, while the PES membrane

is at the distal end with a considerably lower temperature. We employed temperature indicating strips to measure the temperatures at various locations. The results demonstrated that the temperature at the position where the PES membrane was placed ranged from 77 to 82°C, falling within the tolerable range of the PES membrane. Meanwhile, the SEM images of the TbPa/PES membrane and Cu₂O/TbPa/PES membrane indicated that such temperature has no impact on the structure of the PES membrane. We have incorporated the following results and text into the revised manuscript:

“The temperatures at various locations within the tube were measured using temperature-indicating strips (Supplementary Figure 1). The results demonstrated that the temperature at the location of the PES membrane ranged from 77 to 82 ° C, falling within its tolerance range.”



Supplementary Figure 1. The temperatures at various locations within the tube.

(3) The reviewer's suggestion to merge Figures 2 and 3 for potential clarity has been considered. However, due to the complexity and detail in Figures 3, incorporating the content of Figures 2 could result in an unbalanced visual presentation. Attempts to adjust the figures accordingly did not yield a more favorable outcome. As a result, we have chosen to maintain the figures as separate entities in the revised manuscript.

The adjusted Figure 2 is as follows:

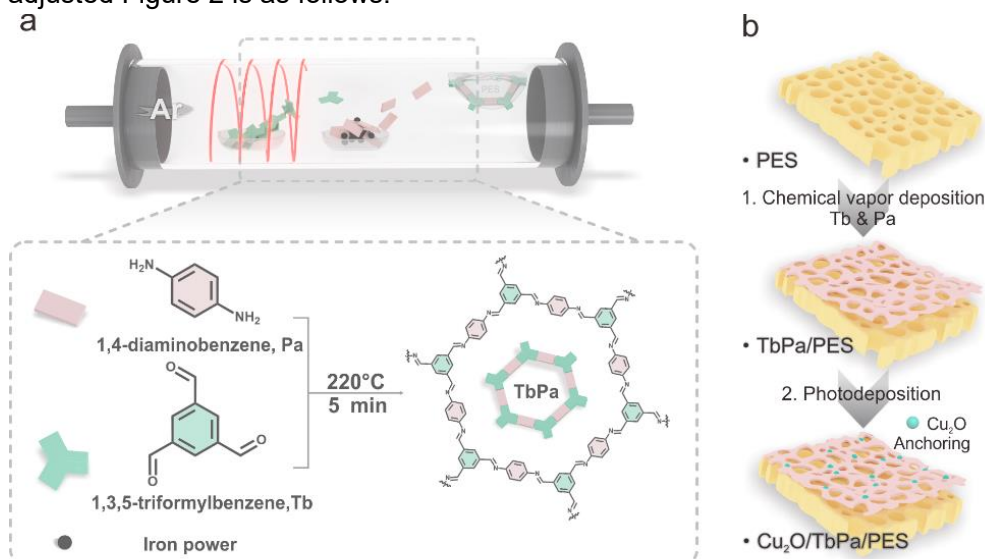


Figure 2. Synthesis of Cu₂O/TbPa/PES membranes. (a) Schematic diagram of vapor deposition of TbPa in a tubular furnace. (b) Schematic diagram of the synthesis of Cu₂O/TbPa/PES membranes.

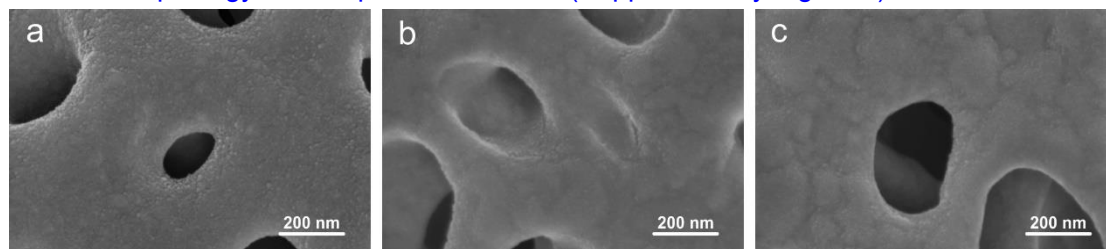
Comment 4. Line 124-125 on Page 8, ‘... a minimal amount of copper is incorporated onto TbPa’. Is there any quantitative data available?

Response: Yes, the copper content was obtained by ICP-MS testing, and the specific values are given in Supplementary Table 1.

Comment 5. Figure 3g on Page 10, what is the morphology of TbPa COFs in the SEM image?

Response: Following this suggestion, we have examined the PES membrane, TbPa/PES membrane, and Cu₂O/TbPa/PES membrane with high-resolution SEM. The results show that the deposited TbPa presents as a thin film conformally adhered to the PES substrate. We discussed this point in the revision as below:

“Moreover, high-resolution SEM images indicate that the deposited TbPa presents as a thin film conformally adhered to the PES substrate, and there is no noticeable change in surface morphology after deposition of Cu₂O (Supplementary Figure 4).”



Supplementary Figure 4. High-resolution SEM images of (a) the PES membrane, (b) the TbPa/PES membrane, and (c) the Cu₂O/TbPa/PES membrane.

Comment 6. Line 185 and 187 on Page 11, why do Figure 3j and 3k demonstrate the regular and ordered anchoring of Cu₂O clusters? Do they show a periodic arrangement?

Response: Figure 3j displays a HAADF-STEM image of Cu₂O/TbPa. The brightness of the image directly reflects the square of the atomic number, making it possible to distinguish Cu elements from C, N, and O elements in TbPa. It is observed that the bright spots are evenly distributed without any significant aggregation, demonstrating that the Cu₂O clusters of nanoscale size exhibit a uniform distribution throughout TbPa. The favorable distribution of these clusters is attributed to the formation of ordered redox-active sites within the COF under illumination, which serve as anchoring sites for the Cu₂O clusters. Furthermore, the energy dispersive spectroscopy (EDS) scan results presented in Figure 3k provide support for the uniform distribution of Cu elements. However, it is important to note that, based on current results, this distribution pattern does not exactly adhere to ‘periodic arrangement’. In recognition of this, we have made appropriate adjustments to the manuscript to ensure a more accurate portrayal of experimental observations. The results and discussions were revised as follows:

“The visualizations indicate that the Cu₂O clusters, measuring approximately 0.25 nm in diameter, exhibited a uniform dispersion pattern on the TbPa surface.”

Comment 7. Line 211-212 on Page 12, what does the bandgap energy of TbPa (~ 2.52 eV) mean?

Response: The bandgap energy of TbPa, measured to be approximately 2.52 eV, signifies

the energy difference between the bottom of the conduction band and the top of the valence band in TbPa. This is crucial as it determines the wavelength of light that can excite electrons from the valence band to the conduction band. Understanding the bandgap energy of TbPa is therefore essential for elucidating its semiconductor characteristics and evaluating its potential applications in photocatalytic sterilization.

Comment 8. *What is the 'LZU1' in Figure 5e, 5f, and 5h?*

Response: We thank the reviewer for pointing out this issue. LZU1 is an alternative name for TbPa COF, and we acknowledge the importance of maintaining a consistent name throughout the text. We used TbPa throughout the manuscript in the revision including Figures 5e, 5f, and 5h as mentioned by the reviewer.

Comment 9. *Figure 7e on Page 28, why don't the dead bacteria clog the membrane? After bacterial lysis, could toxic intracellular contents leak into the filtrate?*

Response: In response to the first question, dead bacteria are less likely to clog the membrane compared to live bacteria because they have lost their ability to adhere and colonize on surfaces. Additionally, the reactive oxygen species generated in our system continuously disrupt the phospholipid bilayer of bacterial cell membranes, effectively inhibiting the adhesion of dead cells to the membrane surface.

Regarding the second question, a certain amount of endotoxin may be released after bacterial lysis. However, downstream water treatment technologies such as reverse osmosis can effectively remove these endotoxins if necessary, thereby significantly reducing any potential hazards.

Comment 10. *Line 515-516 on Page 28, 'it excels in membrane separation performance'. This sentence is confusing, what substances can be separated using this membrane? Are there any experimental data available?*

Response: There might be a misunderstanding. This sentence is to highlight the membrane's exceptional performance in terms of permeability, sterilization efficacy, and resistance to fouling, compared to currently advanced filtration membranes. To substantiate this claim, we rephrased this sentence in the revision with reference to experimental results as below:

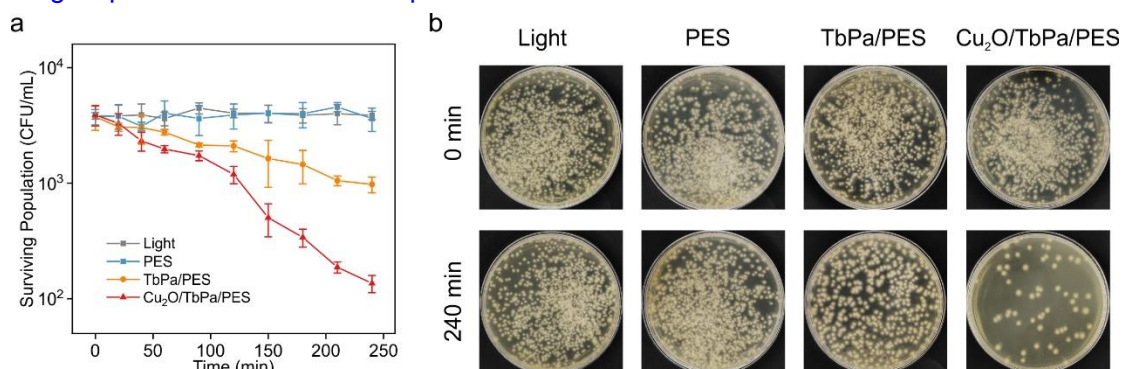
"Specifically, it demonstrates exceptional permeation flux, while also exhibiting significant advantages in antibacterial efficacy and resistance to bacterial fouling (Figure 7d)."

Comment 11. *Can this membrane kill harmful fungi?*

Response: Following this very insightful suggestion, we have investigated the performance of our membranes killing fungi. Fungi, particularly molds, are ubiquitous organisms characterized by their compact and intricate spore structures, which endow them with robust resistance against external stressors. It is a common pathogen in polluted water bodies. Therefore, we selected *Aspergillus niger* (CMCC 98003) spores as a model to test the fungicidal ability of the Cu₂O/TbPa/PES membrane. We have supplemented the relevant content on page 21 as follows:

"Furthermore, the results depicted in Supplementary Figure 1 reveal that the

Cu₂O/TbPa/PES membrane achieves approximately a 1.5-log reduction in *Aspergillus niger* spore colony counts within 4 hours, indicating its promising application in addressing fungal spore contamination in aquatic environments.”



Supplementary Figure 7. (a) Survival curves of *Aspergillus niger* spores following treatment with PES, TbPa/PES and Cu₂O/TbPa/PES membranes at various illumination times. (b) Plate spread photographs of *Aspergillus niger* spores after treatment with different membranes and their respective control groups.

We have also supplemented the methods for the antifungal test in the Supporting Information as follows:

“Anti-fungal tests

Aspergillus niger (CMCC 98003) was inoculated onto Sabouraud dextrose agar plates and incubated at 37°C for 5 d. Subsequently, spores were collected using sterile NaCl solution and filtered through eight layers of sterile gauze to remove any hyphae. The spore suspension was then adjusted to a concentration of 10⁴ CFU mL⁻¹. The subsequent inactivation test followed the same methodology as the anti-bacterial tests.”

The response to reviewer' comment

Manuscript ID: NCOMMS-24-70322B

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Reviewer #1:

The authors have made appropriate corrections to the comments of manuscript. The modified and added contents are highlighted. This manuscript is suitable for publication in the present form.

Response: We are grateful to the reviewer for their positive feedback and the support of the publication of this work.

Reviewer #2:

The authors have properly revised the manuscript.

Response: We appreciate the reviewer's comments about the quality of our study.