Supporting Information

Visualization and assessment of the microbial colonization process of disposable surgical mask in typical natural aquatic environment

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This Supporting Information contains 9-pages of text, one tables and six figures, including this cover page.

1. Image analysis

The fluorescence images of the biofilm-colonized disposable surgical mask pieces stained by LIVE/DEAD® BacLight™ bacterial viability kits were analyzed by using the open-source software Fiji (version 1.53c, Wayne Rasband, National Institutes of Health, USA).[1] The image analysis procedures are as follows (Figure S4):

(1) Input the fluorescence images; (2) Change the image type of all analyzed images to 8-bit gray scale and subtract the background by setting rolling ball radius to 50 pixels; (3) Sementation, choose 'default' option and adjust threshold value to get the appropriate image; (4) Use binary and watershed to make the segment more accurate; (5) Analyze particles, remove the particles that are too small by setting the smallest size of 0.3 μm^2; (6) Output.[2-4]

2. Cell abundance calculation

The cell abundance of the biofilm colonized disposable surgical mask was quantified by A%, which was defined as the percentage of microorganisms colonized area to the whole image area; the microorganisms colonized area of each fluorescent image was calculated following the image analysis procedures in Figure S4. Then, A% was defined as:

$$A\% = A_{\text{Micoorganisms colonized area}} / A_{\text{Fluorescent image area}}$$

Additional Literature Cited

(1) Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J. Y.; White, D. J.; Hartenstein, V.; Eliceiri, K.; Tomancak, P.; Cardona, A., Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **2012,** *9*, (7), 676-682.

(2) Shihan, M. H.; Novo, S. G.; Le Marchand, S. J.; Wang, Y.; Duncan, M. K., A simple method for quantitating confocal fluorescent images. *Biochem. Biophys. Rep.* **2021,** *25*, No. 100916.

(3) Zhou, Z.; Tan, Y.; Xiao, Y.; Stuckey, D. C., Characterization and significance of sub-visible particles and colloids in a submerged anaerobic membrane bioreactor (SAnMBR). *Environ. Sci. Technol.* **2016,** *50*, (23), 12750-12758.

(4) Bera, T.; Xu, J.; Alusta, P.; Fong, A.; Linder, S. W.; Torosian, S. D., Estimating bacterial concentrations in fibrous substrates through a combination of scanning electron microscopy and ImageJ. *Anal Chem* **2019,** *91*, (7), 4405-4412.

Table S1 Identification of main absorbance peaks in FTIR spectra of disposable surgical mask exposed in Nanhu Lake at Site 1.

Wavelength (cm^{-1})	Description
3400-3200	O-H and N-H stretching vibration
2948	Asymmetric stretching vibration of CH_3
2915	Asymmetric stretching vibration of CH_2
2868	Symmetric stretching vibration of CH_3
2837	Symmetric stretching vibration of CH_2
1648	Amide Ⅰ: C=O stretching, N-H bending and C-N bending
1553-1535	Amide Ⅱ: C-N and N-H bending, C-N stretching
1452	Asymmetric bending of CH_3 and bending vibration of CH_2
1374	Symmetric bending of CH_3
1245-1210	C-N stretching and N-H bending; C-O stretching; P=O stretching in phosphates
1100-1048	C-N stretching; C-O-C, C-OH vibrations in polysaccharides

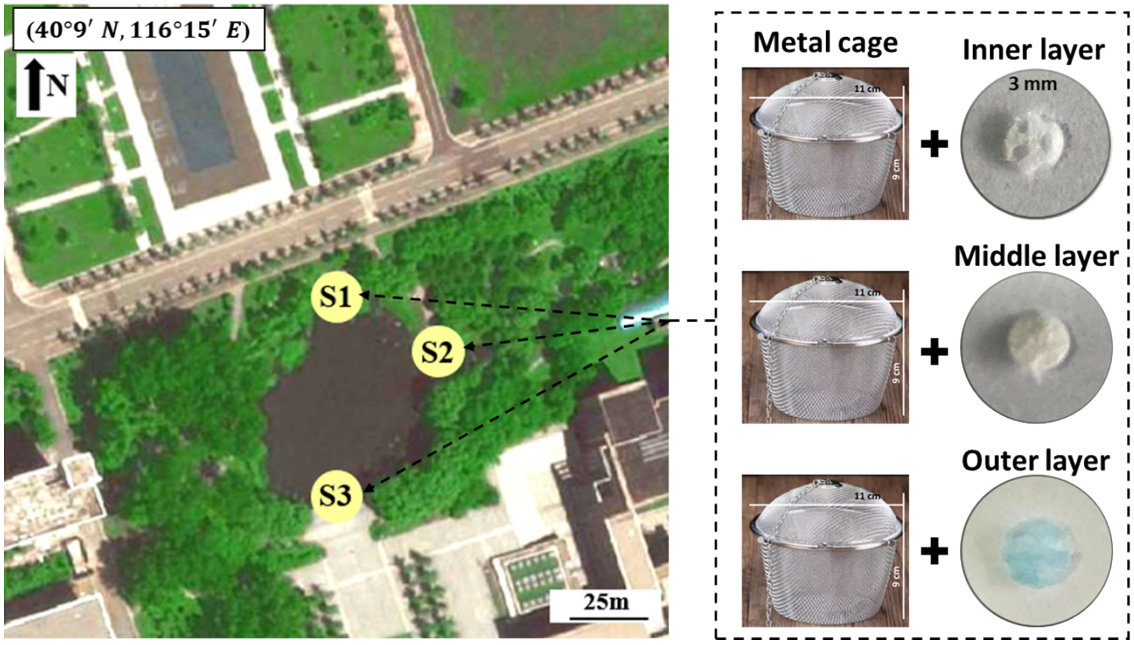


Fig. S1 The spatial location of DSM exposure sites and the experimental setup in this study.

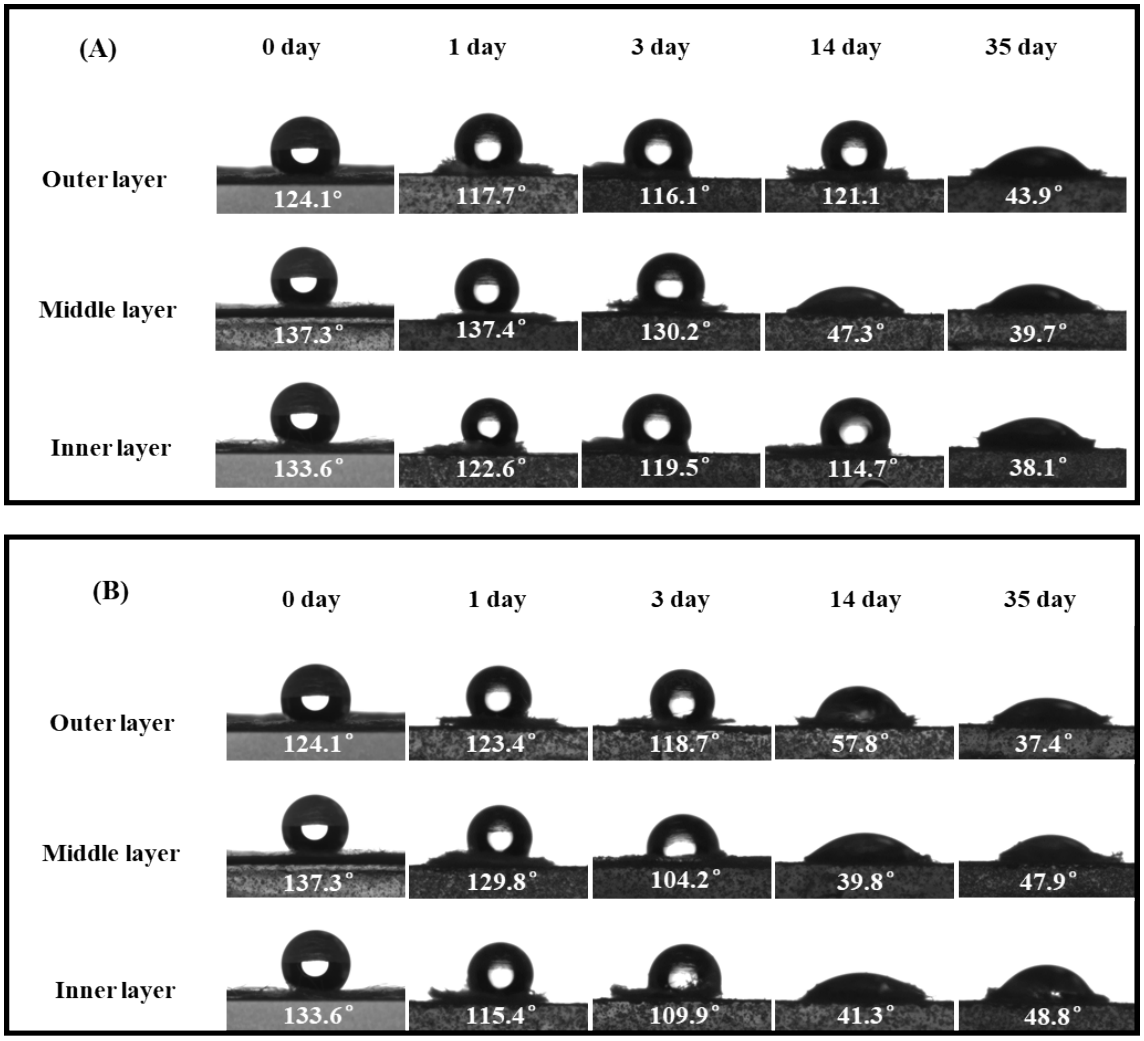


Fig. S2 Contact angle of the biofilm colonization process on disposable surgical mask after 1, 3, 14, 35 days of exposure in Nanhu Lake at (A) Site 2 and (B) Site 3.

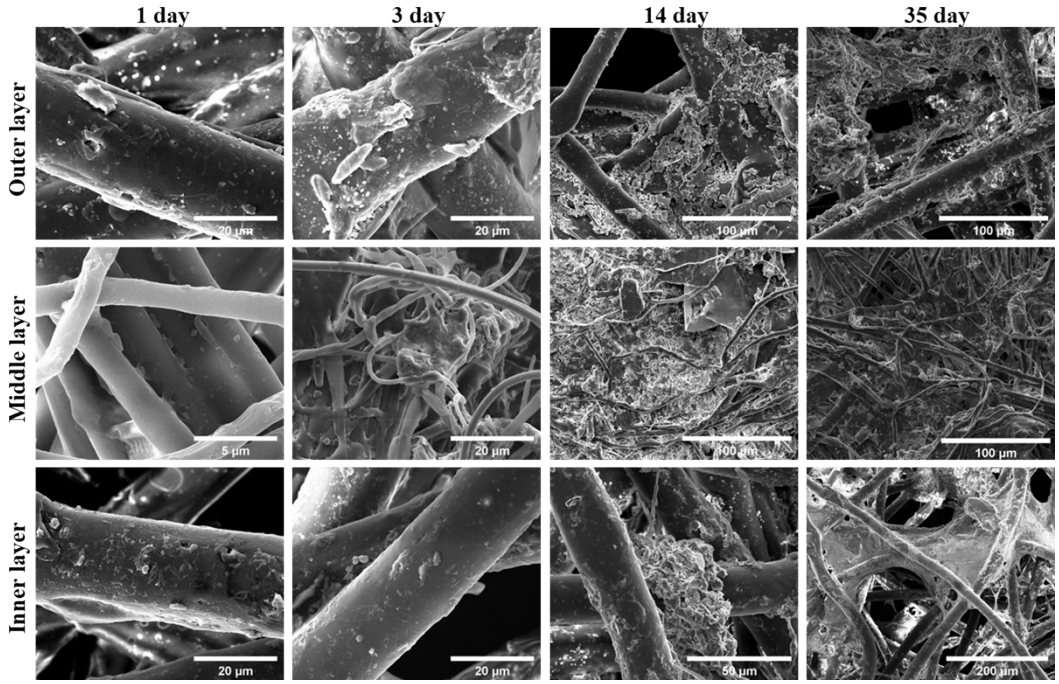


Fig. S3 The magnified SEM images of different layers of the disposable surgical mask.

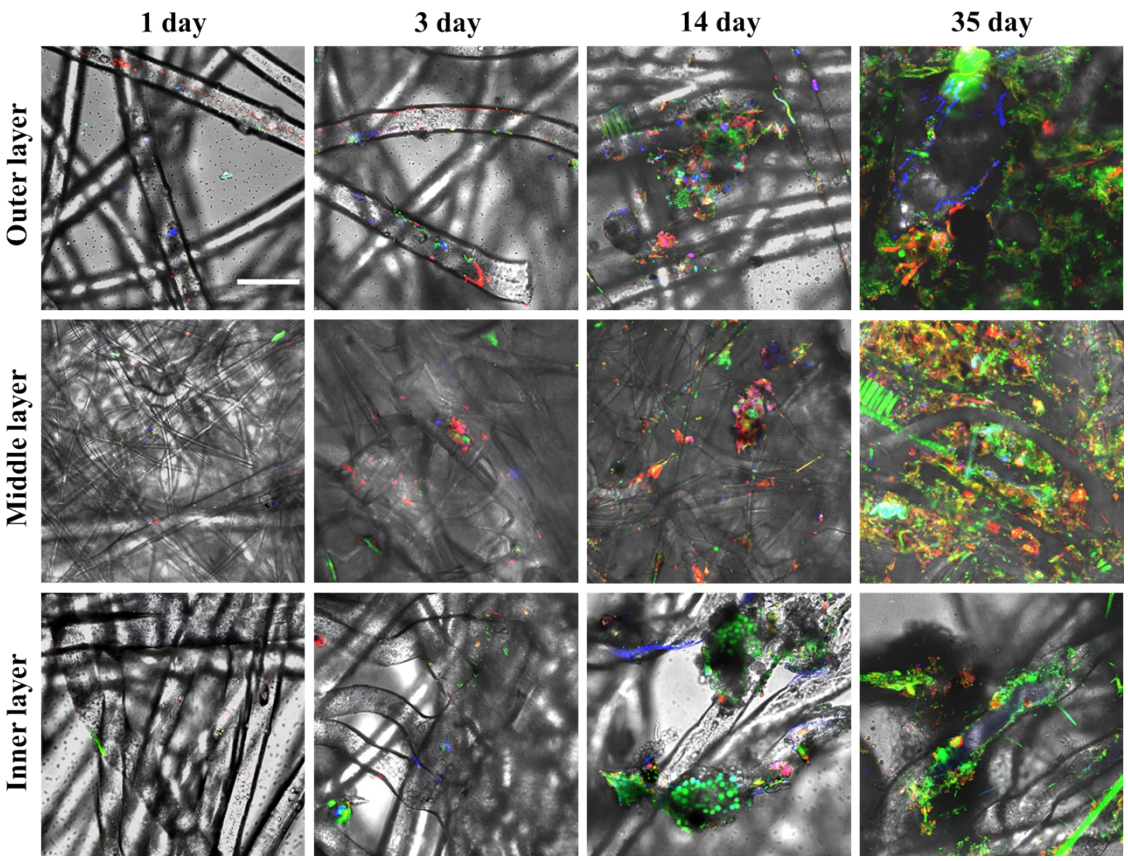


Fig. S4 CLSM images of the biofilms colonized DSM after 1, 3, 14 and 35 days of exposure in Nanhu Lake at Site 1. Color allocation: blue (cells), green (protein), red (α-D-glucopyranose polysaccharides), and transmission image of black and white. (scale bar 50 μm)

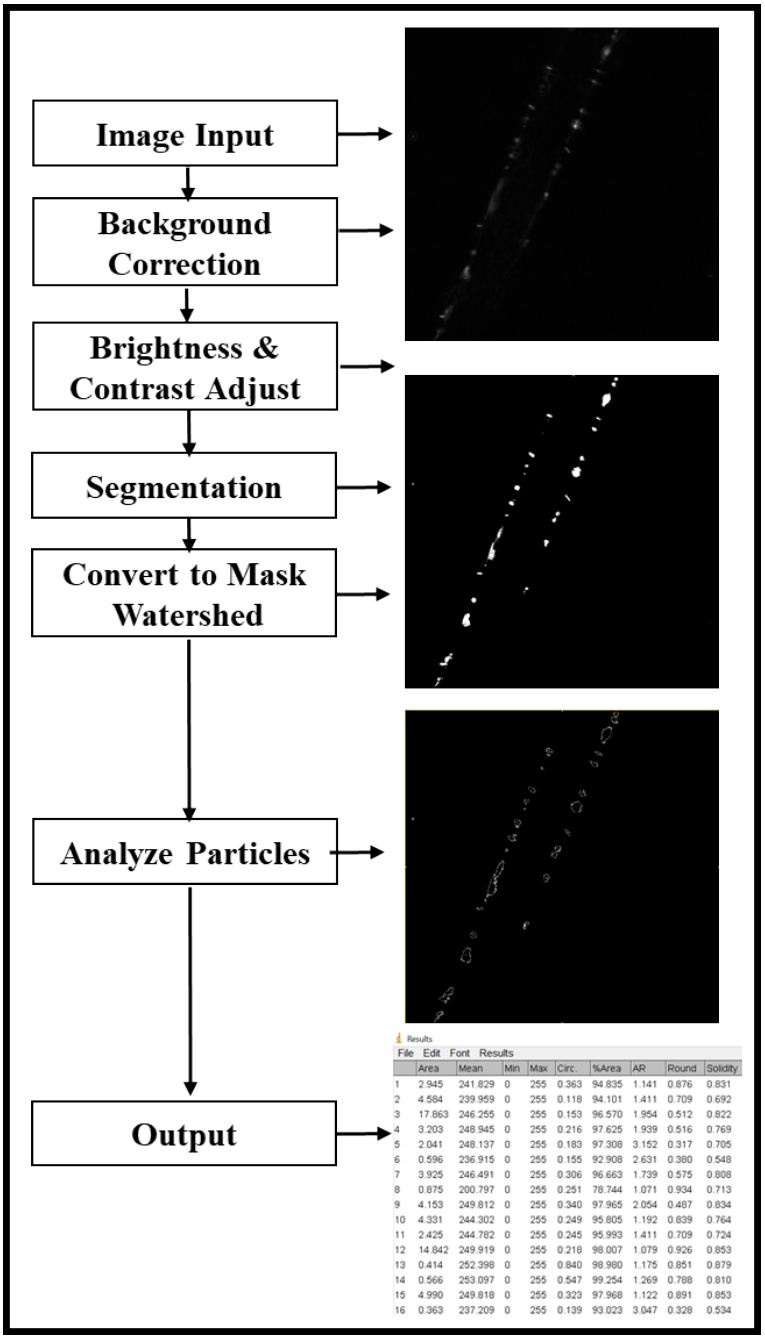
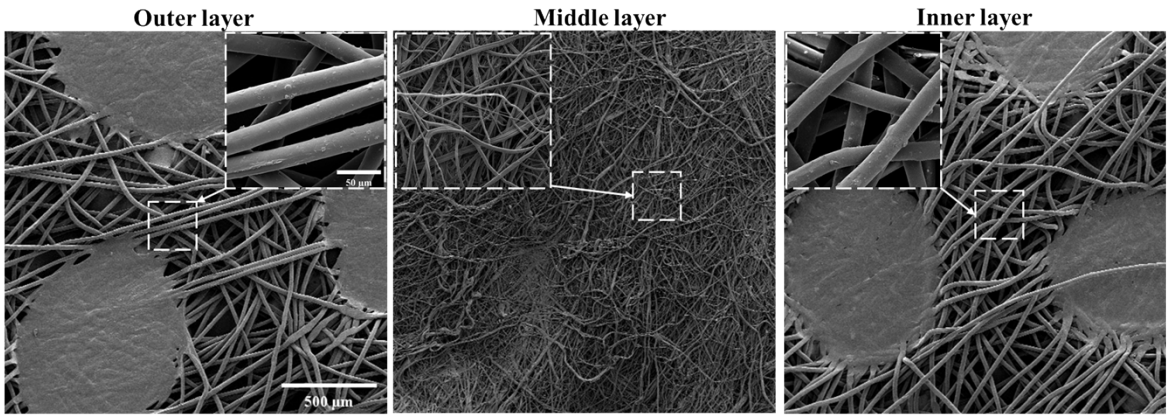


Fig. S5 Schematic representation of the fluorescence image analysis procedure.



Layer	Diameter of DSM fibers (μm)	Porosity of DSM debris (%)
Outer layer	21.37±2.20	12.36±3.60
Middle layer	3.12±0.88	4.81±0.74
Inner layer	21.28±1.79	8.88±1.50

Fig. S6 Surface characteristics of different layers of the disposable surgical mask.