Supporting information

Text S1. Surface layer imaging.

This study utilized scanning electron microscope (SEM) and laser scanning confocal microscope (LCSM) for surface layer imaging. The pretreatment of the fouling layer involved the following steps: 4% paraformaldehyde solution was used to fix the membranes for 24 h, washed three times using 0.1 mol/L PBS. Subsequently, the membranes were dehydrated through a series of alcohol solutions (50%, 75%, and 100%, 5 min per step). After coating with platinum, the fouling layers were observed by SEM (INSPECT S50, Thermo Fisher, USA).

Fluorescein isothiocyanate (FITC, Thermo Fisher, USA) and Calcofluor white (Sigma, Germany) were used to stain proteins and β-D-glucopyranose polysaccharides in different surface layers. After each stain, the sample was washed three times with PBS to remove any excess stains. For sample pretreatment and detailed operating procedures, please refer to (Chen et al. 2006, Lei et al. 2021). LCSM (CSSP8, Leica, Germany) was employed to capture the images of above substances, and excitation and emission widths of each stain were set as per the manufacturer's instructions.

Text S2. The EEM spectra analysis of EPS profile.

The mixture of fouling on the membrane surface was centrifuged, and the supernatants were filtered through 0.45 μm PES membrane, and the filtrates were SMP. The residual solids were extracted by hot extraction method to extract LB-EPS and TB-EPS. Before analysis, the samples were diluted with pure water and adjusted to pH 3.0 using hydrochloric acid. Three-dimensional fluorescence excitation-emission matrix (EEM) was performed to characterize SMP and EPS compositions using a luminescence spectrometer (F-7000, Hitachi, Japan) with background data normalized using the Raman peak of pure water (Jacquin et al. 2017).The excitation and emission wavelength ranges were set to 200–500 nm and 250–600 nm at intervals of 2 nm.

Text S3. Sample preparation for metagenomic analysis.

The suspension derived from fouling layer and sludge were added cold PBS up to 40 mL, vortexed, and sonicated to detach bacteria and viruses. Subsequently, the mixtures were centrifuged at 4500 g for 20 min to isolate supernatants. The precipitates were resuspended and the above extraction process was repeated thrice. Mixed the extracted supernatant and collected bacteria and virus on membrane based on Zang's methods (Zang et al. 2024). The total microbial DNA was extracted using E.Z.N.A.® Viral DNA Kit (OMEGA, USA). For viral DNA extraction, the virus concentration was treated with DNase I (Thermo Scientific, USA) to remove nonencapsulated DNA fragments and then extracted DNA using E.Z.N.A.® Viral DNA Kit (OMEGA, USA). Shotgun sequencing were performed on Illumina NovaSeq platform (Illumina Inc., USA) to obtain 150 bp paired-end reads.

Table S1 Primary characteristics of sludge and domestic sewage.

Sample	Parameter	Concentration (mg/L)
Sludge	Total solids (TS)	10.0 – 10.6
	Volatile solids (VS)	8.0 – 8.5
Sewage	Total chemical oxygen demand (TCOD)	189.6 – 410.9
	Total nitrogen (TN)	33.8 – 58.5
	Total phosphorus (TP)	3.4 – 10.3
	pH	7.8

Table S2 Operating conditions of AnMBR.

Operation conditions	Values
Working volume (L)	2
Temperature (°C)	25
HRT (h)	6.7
OLR (g COD/L/d)	0.6 – 1.4
Flux (L/m^2 h)	8.2
Influent cycle	30s on and 2.5 min off
Effluent cycle	1 min on and 2 min off

Table S3 Raw and assembly summary for bacterial metagenomes.

Parameter	C1	C2	C3	G1	G2	G3	N1	N2	N3
Raw reads	104054386	67182094	81368818	107501020	67238018	77902412	89315020	69320424	69346162
Clean reads	100945616	66985230	77405656	104155730	67066622	74207930	87323674	65716732	65793716
Q20 (%)	99.10	98.85	98.78	99.06	99.04	98.78	98.95	98.73	98.66
GC (%)	48.03	48.87	48.65	47.15	48.58	47.99	51.62	48.25	47.62
Contig number	423102	433704	295489	415997	395771	257158	517670	253933	267853
Contig length	559700510	591501720	370308420	535485746	499244600	319123328	722532356	363196585	365849236
N50	1617	1671	1429	1514	1435	1409	1803	1854	1727
N90	589	599	585	589	586	586	598	601	591
Max length	466462	466123	211146	529347	372617	202014	840322	687893	571095
Min length	500	500	500	500	500	500	500	500	500
Contig number (>1 kb)	137091	147822	95048	135259	125244	83341	173163	85160	86231
Prophage contigs	13292	12993	11001	24385	22621	20567	5998	4829	4311

Table S4 Raw and assembly summary for viral metagenomes.

Parameter	C1	C2	C3	G1	G2	G3	N1	N2	N3
Raw reads	152016824	100700750	138125220	160286784	98625294	172421066	170676170	151929670	139752174
Clean reads	149305858	95216574	131372408	157470612	93449610	164185406	166302150	144571170	132893504
Q20 (%)	99.20	99.17	98.75	99.19	99.26	98.85	98.82	98.73	98.83
GC (%)	51.61	52.22	48.36	50.06	46.94	48.75	51.56	50.87	50.52
Contig number	746233	581698	356102	748690	406282	453903	749962	380805	418409
Contig length	1021328019	775084096	424898733	993072327	510224502	543239438	1075002558	444835705	499290622
N50	1710	1601	1327	1605	1442	1326	1861	1236	1281
N90	595	597	572	597	584	580	608	568	570
Max length	948196	840365	265810	529270	464350	566723	619869	1033167	687893
Min length	500	500	500	500	500	500	500	500	500

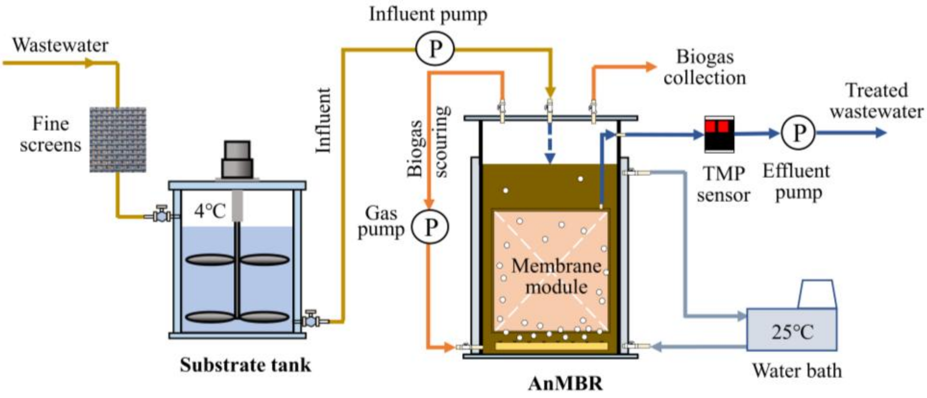

Fig. S1 Diagram of submerged anaerobic membrane bioreactor of the study.

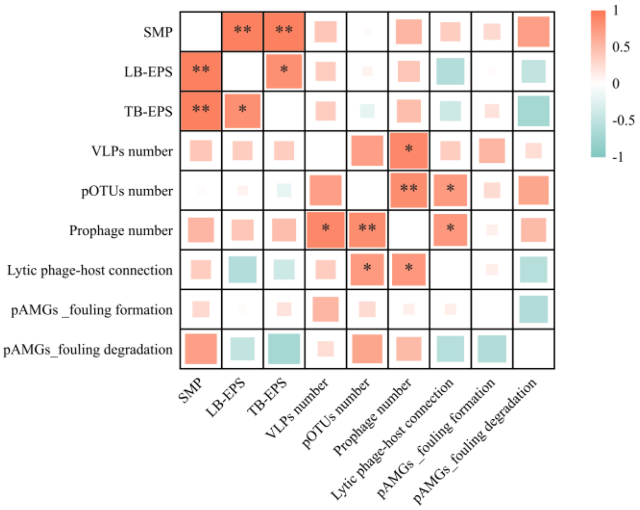

Fig. S2 Correlation matrices (Spearman correlation) of virus and EPS components.

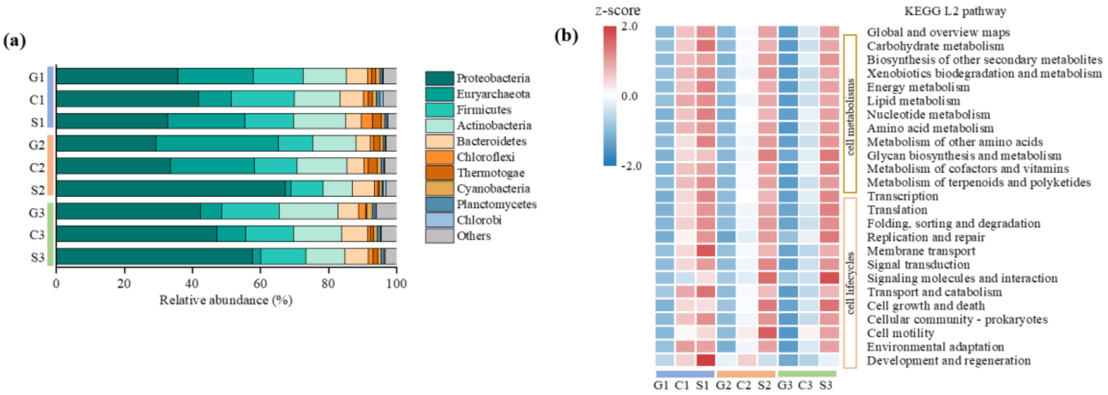

Fig. S3 Bacterial communities and KEGG functional annotations in membrane fouling and sludge. (a) The relative abundance of bacteria phyla. (b) Heatmap showed the relative abundance of KEGG level 2 pathways. The blue, orange, and green lines correspond to stage 1, 2, and 3, respectively.

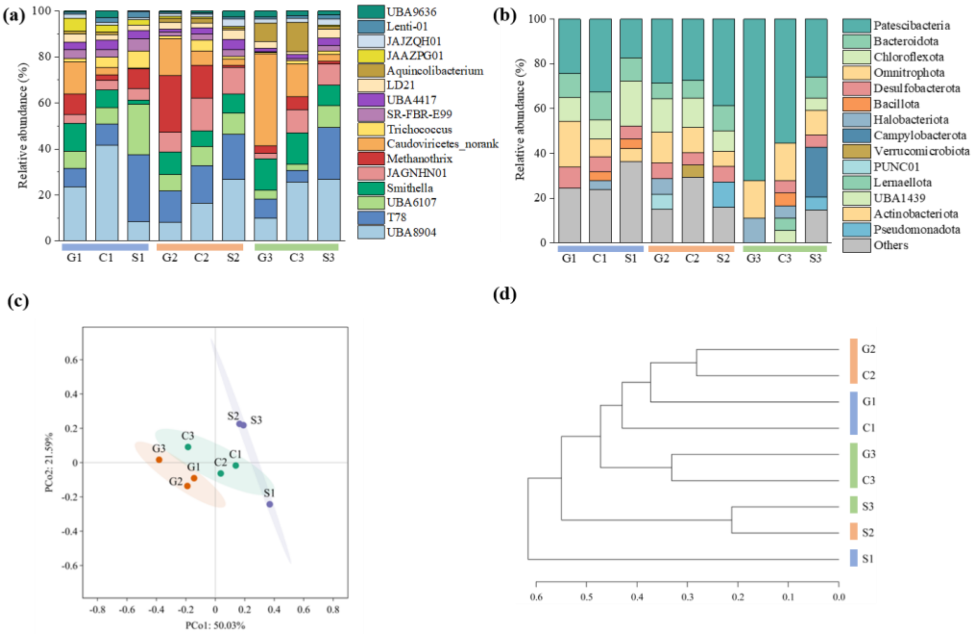

Fig. S4 Difference in bacterial communities between sludge and membrane fouling based on contigs and MAGs taxonomy. (a) Relative abundance of major bacteria genus based on contigs taxonomy. (b) Relative abundance of major bacteria phyla based on MAGs taxonomy. PCoA analysis (c) and Hierarchical clustering analysis (d) comparing differences in community composition.

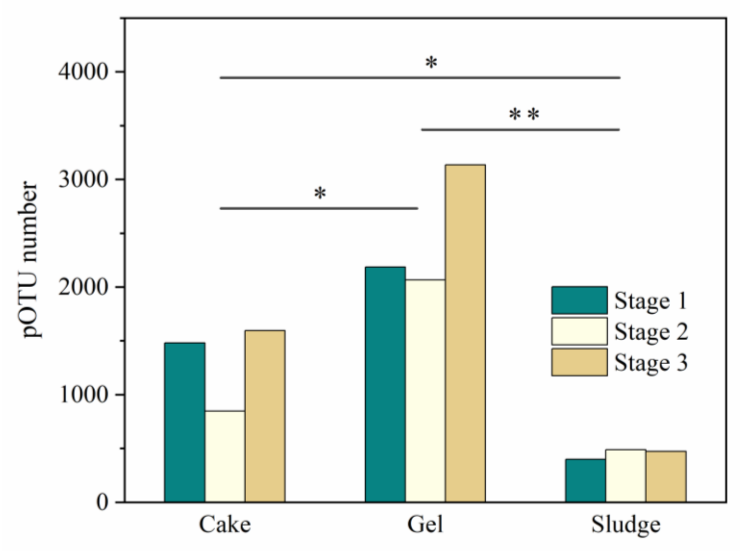

Fig. S5 The numbers of pOTUs in the cake layer, gel layer, and sludge.

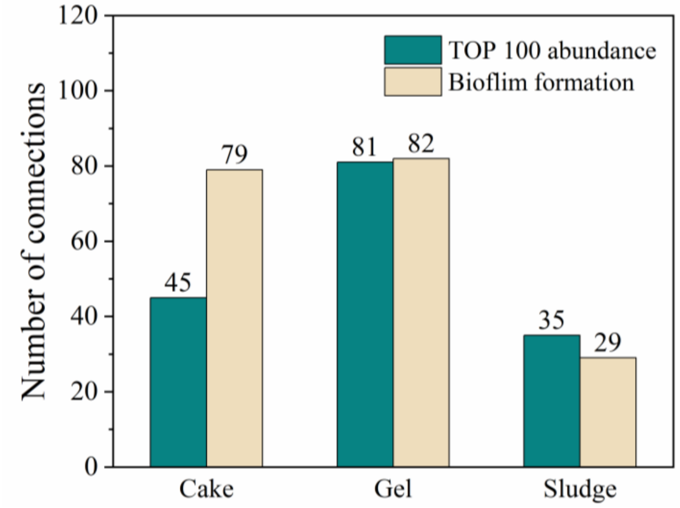

Fig. S6 Number of lytic phage-host connections associated with the top 100 abundant species and biofilm formation in the cake layer, gel layer, and sludge.

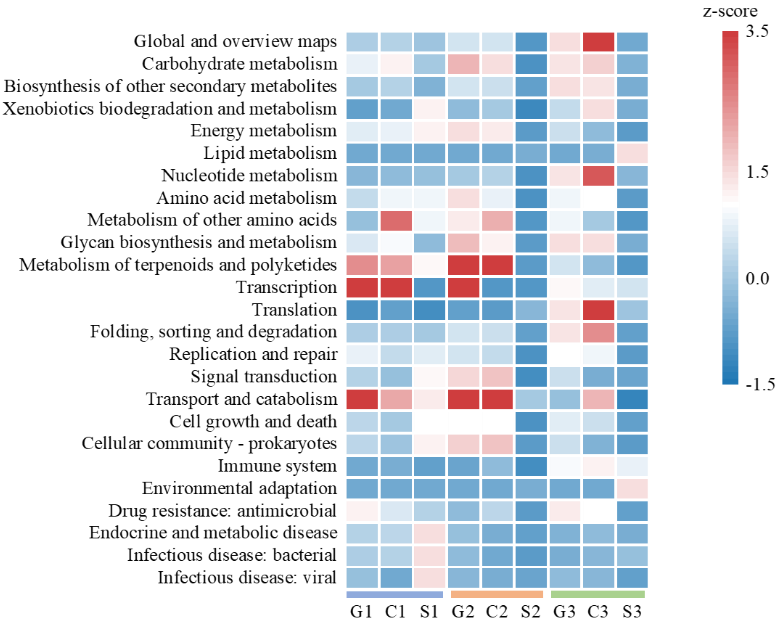

Fig. S7 Heatmap analysis of phage-encoded AMGs annotated to concerned KEGG level 2 pathways.

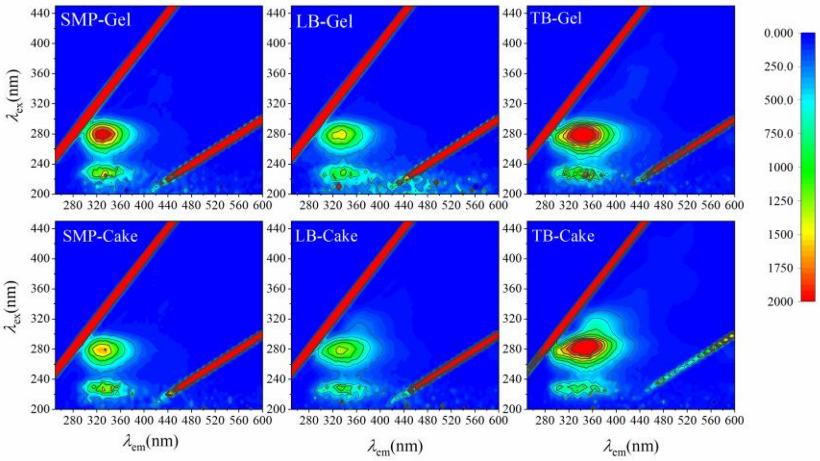

Fig. S8 EEM spectra analysis of SMP and EPS profiles in membrane fouling. LB: loosely bound EPS, TB: tightly bound EPS.

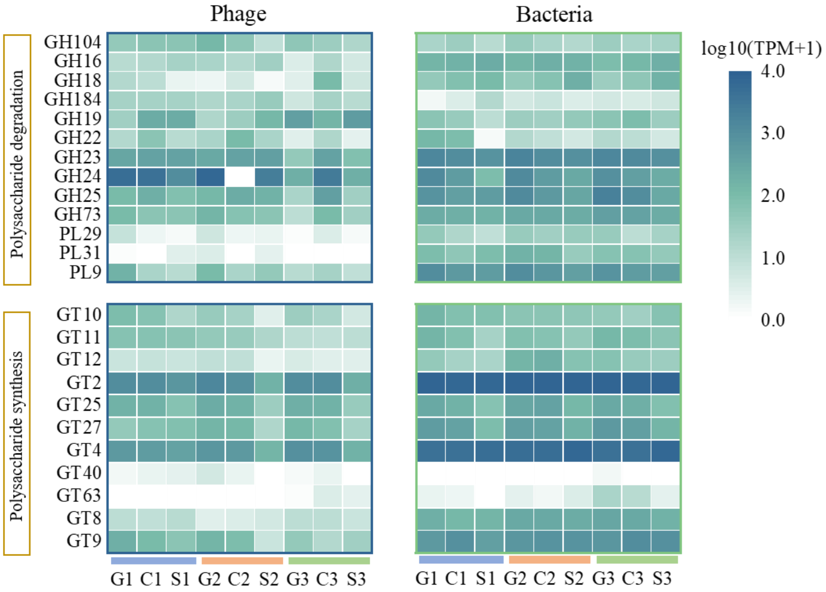

Fig. S9 Heatmap analysis of phage-encoded AMGs annotated to CAZy family related to polysaccharide synthesis and degradation.

Supplementary references

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