Supporting Information

Removal of antibiotic-resistant bacteria and antibiotic resistance genes affected by varying degrees of fouling on anaerobic microfiltration membranes

Hong Cheng§, Pei-Ying Hong§*

§ King Abdullah University of Science and Technology (KAUST), Division of Biological and Environmental Science & Engineering (BESE), Water Desalination and Reuse Center (WDRC), Thuwal, 23955-6900, Saudi Arabia

Summary of contents

Supporting Information – 15 pages

Includes: Supporting methodology – 3 pages
Supporting tables – 2 tables
Supporting figures – 8 figures

* Corresponding author:

Pei-Ying Hong

Email: peiying.hong@kaust.edu.sa

Phone: +966-12-8082218

1. Growth conditions for antibiotic-resistant bacteria Single colonies of *E. coli* PI-7, *K. pneumoniae* L7 and *E. coli* UPEC-RIY-4 were respectively inoculated into 500 mL LB Broth (Lennox) (Sigma-Aldrich, St. Louis, MO, US) that contained the corresponding antibiotics. 25 μg/mL meropenem (Sigma-Aldrich, Buchs, Switzerland) was added to the LB inoculated with *E. coli* PI-7, while 8 μg/mL ceftazidime was added to the LB inoculated with either *K. pneumoniae* L7 or *E. coli* UPEC-RIY-4. The suspensions were incubated in a 200 rpm shaker incubator for 17 h at 37 ^{o}C. After this, the cultures were harvested by centrifuging at 10,000 g for 10 min, and the pellets were resuspended and washed by 1X M9 Minimal Salt medium twice prior to filtration experiment. This procedure was performed to prevent any bacterial regrowth from occurring during the filtration experiment.

2. Plasmid sequencing and assembly Single colony of *K. pneumoniae* L7 and *E. coli* UPEC-RIY-4 were respectively inoculated into 10 mL LB broth with 8 μg/mL ceftazidime (Sigma-Aldrich, Buchs, Switzerland), and incubated in a 200 rpm shaker incubator for 17 h at 37 ^{o}C. The cells were then harvested by centrifuging at 10000 g for 10 min, and the cell pellets were extracted for plasmids by PureYield^{TM} Plasmid Miniprep System (Promega, Madison, WI, USA). 200 ng of each plasmid was prepared for its DNA library by TruSeq$^{®}$ Nano DNA LT Library Prep Kit and sequenced via Illumina Miseq platform (Illumina, San Diego, US). Raw sequences were preprocessed and checked for quality using Trimmomatic v.0.3.3. The raw sequences were respectively mapped on the associated genomes (i.e., *K. pneumoniae* and *E.coli*) to remove genomic DNA contaminant. The filtered data was further assembled by Spades v3.9.0 with kmer sizes 21, 33, 55 and 77. The resulting contigs were mapped over a reference plasmid to further assemble into scaffolds. Based on the scaffolds, the anticipated size of plasmid encoding $bla_{\text{OXA-48}}$ should be larger than or equal to 55 Kb. The anticipated size of plasmid encoding $bla_{\text{CTX-M-15}}$ should be larger than or equal to 110 Kb [1]. According to previous study, the size of plasmid encoding $bla_{\text{NDM-1}}$ was about 110 Kb [2].The plasmid sequencing data generated in this study was submitted to the European Nucleotide Archive (ENA) under study accession number PRJEB21923.

3. Real-Time PCR and detection limit test qPCR standards for the respective genes were prepared as described as previously [3]. The qPCR standard curve was produced by diluting in series to obtain concentration ranging from 10^2 to 10^8 copies/μL. Each 20 μL reaction volume consist of 10 μL Taqman fast master mix, 1 μL of each primer (10 μM), 0.8 μL respective probe, 1 μL of DNA template and 6.2 μL molecular grade H_2O. The reactions was conducted on 96-well thermal cycler block. The thermal cycling profile was as follows: 50 °C for 2 min, 95 °C for 20s and 40 cycles of 95 °C for 1 s, 60 °C for 20s. The standard curve and NTC were performed in triplicate, while test amplifications were performed in duplicate. The amplification factor of the standards ranged from 1.86 to 1.99. All the *R*-squared value of curve were >0.97 and all the NTCs have no determinable C_q values.

To further test the detection limit of qPCR, the standard was further diluted in series to form 75, 50, 25, 10 and 5 copies/μL. Same standard curves were performed and these diluted plasmids of known concentrations were tested in triplicate. The results showed that the detections limits for primer pairs targeting $bla_{\text{NDM-1}}$ gene, $bla_{\text{CTX-M-15}}$ gene and $bla_{\text{OXA-48}}$ gene were 25 copies/μL, 100 copies/μL and 50 copies/μL, respectively.

4. Analysis of particulate sizes Single colonies of *E. coli* PI-7, *K. pneumoniae* L7 and *E. coli* UPEC-RIY-4 were respectively inoculated into 20 mL LB Broth (Lennox) (Sigma-Aldrich, St. Louis, MO, US) that contained the corresponding antibiotics as depicted as section 1. One mL of 17 h incubated bacteria were harvested and washed by M9 twice to remove LB broth, and then diluted 100 x by M9 Minimal Salt medium. Six mL of the 17 h incubated bacteria were respectively extracted their plasmids by PureYield™ Plasmid Miniprep System (Promega, Madison, WI, USA). The plasmids were diluted into 1 mL M9 Minimal Salt medium. The particulate sizes of plasmids and bacteria were evaluated by dynamic light scattering (DLS) (Malvern Zetasizer Nano-ZS90, Malvern Instruments). To verify the sizes of ARB with an alternative method, the diluted ARB were respectively dropped onto an aluminum stub. Three nm thick iridium was sputtered onto the surface by K575X Emitech sputter coater (Quorum Technologies,

UK) after bacteria was air-dried. The FEI Nova Nano scanning electron microscope (SEM) was then used to characterize the ARB at 5 kV.

5. Zeta-potential analysis The diluted plasmids and bacteria suspension used for DLS analyses were further quantified for the particle zeta-potential using the Laser Doppler Velocimetry (LDV) technique (Malvern Zetasizer Nano-ZS90, Malvern Instruments).

Table S1. Matrix of conditions used in this study. N.A. denotes not applicable.

Membrane	**Harvested TMP**	**Definition of membrane used in this study**	**Applied filtration TMP (kPa)**	
N0	N.A.	New membrane	N 0-1	N.A.
			N 0-2	
			N 0-3	
			N 0-4	
F1	20	Subcritically fouled membrane	F 1-1	5
			F 1-2	10
			F 1-3	15
			F 1-4	20
F2	40	Subcritically fouled membrane	F 2-1	10
			F 2-2	20
			F 2-3	30
			F 2-4	40
F3	60	Critically fouled membrane	F 3-1	10
			F 3-2	20
			F 3-3	40
			F 3-4	60

Table S2. Primers and fluorogenic probes for specific detection of resistance genes $bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-48}}$.

Bacteria	Genes	Primers	Probes
E.coli PI-*7*	$bla_{\text{NDM-1}}$	Forword: 5'-ATT AGC CGC TGC ATT GAT-3' Reverse: 5'- CAT GTC GAG ATA GGA AGT G-3'	5'-/56-FAM/AGA CAT TCG /ZEN/ GTG CGA GCT GGC GGA /3IABkFQ/-3'
Klebsiella pneumoniae L7	$bla_{\text{CTX-M-15}}$	Forword: 5'-ATC ACK CGG RTC GCC IGG RAT-3' Reverse: 5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3'	5'-/56-JOEN/CCC GAC AGC TGG GAG ACG AAA CGT/36-TAMSp/-3'
E.coli UPEC-RIY-4	$bla_{\text{OXA-48}}$	Forword: 5'-TCA TCA AGT TCA ACC CAA CC-3' Reverse: 5'-ATG CTG ACC GCC AAT-3'	5'-/56-FAM/CCC ACC AGC /ZEN/ CAA TCT TAG GTT CGA /3IABkFQ/-3'

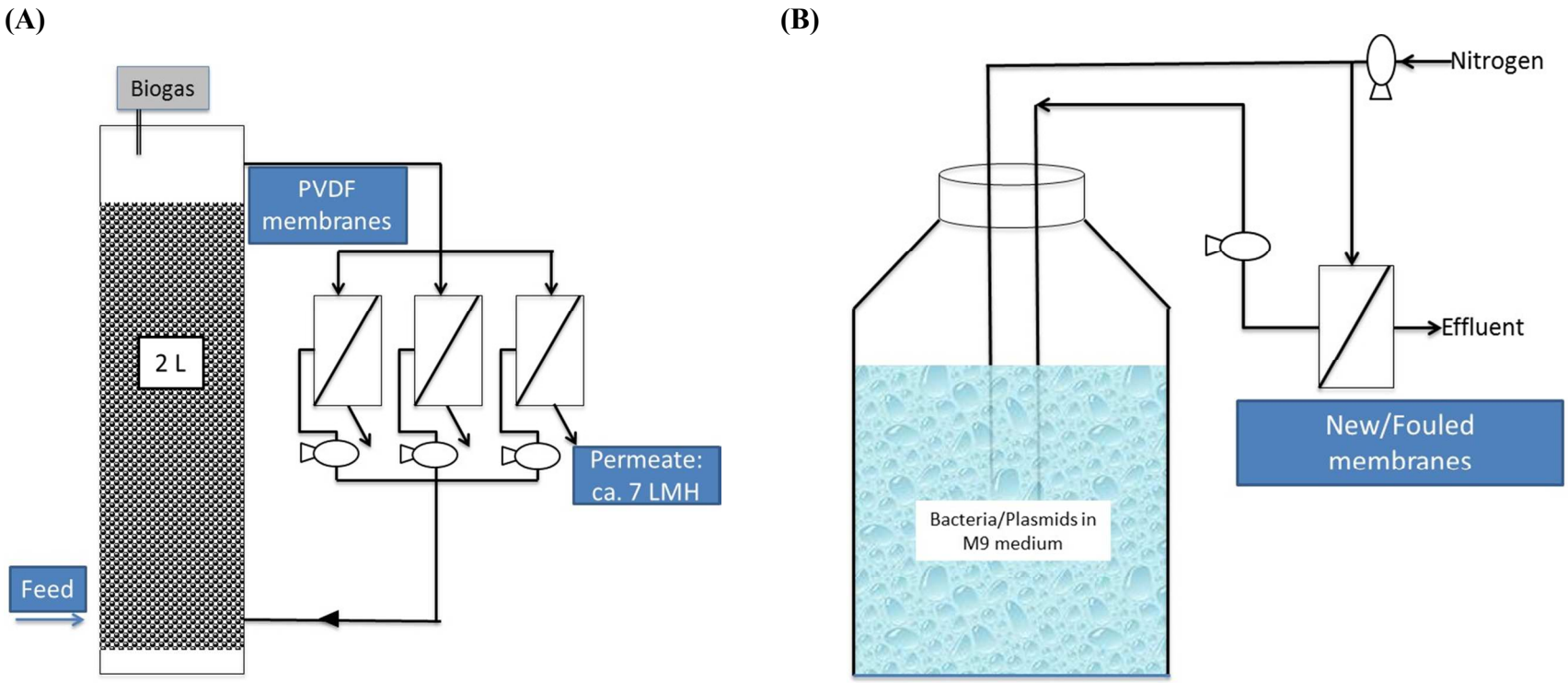

Figure S1. Schematic diagram of anaerobic membrane reactor and experimental setup for ARB and ARGs filtration experiment. **(A)** Reactor configuration and membrane set-up; membranes are individually housed in cassette holders and harvested at different transmembrane pressures **(B)** Membranes housed in the individual cassettes were dismantled from anaerobic reactor and connected to medium containing either ARB or ARGs for subsequent filtration experiments.

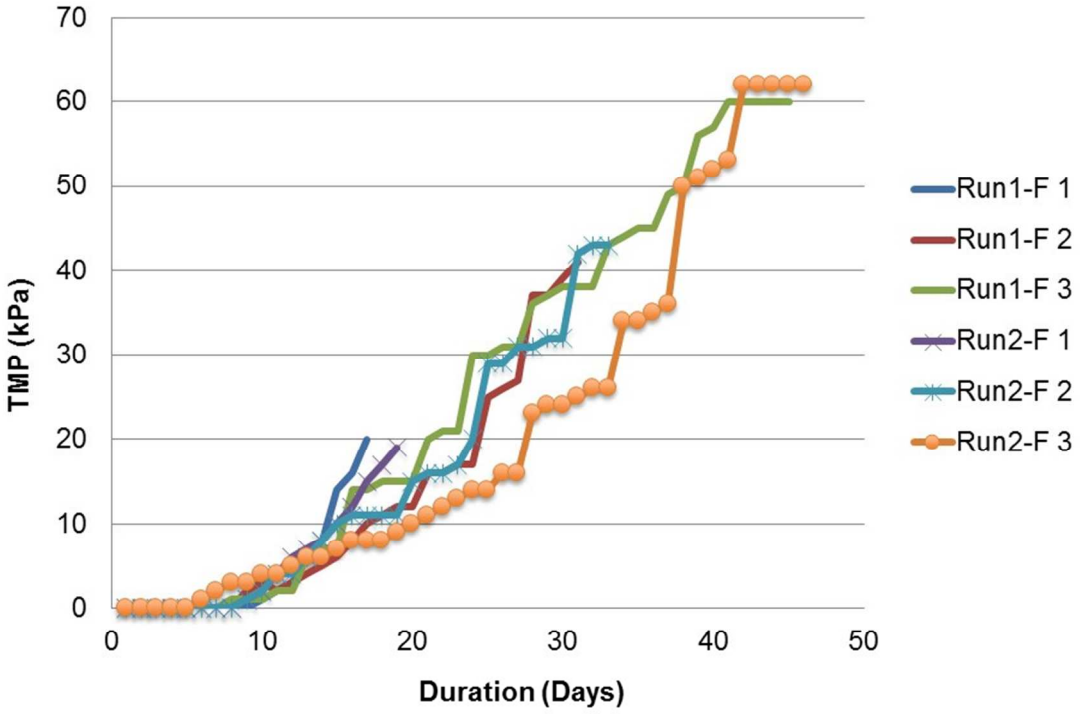

Figure S2. Changes in transmembrane pressure (TMP) for the different PVDF microfiltration membranes connected to anaerobic membrane reactor.

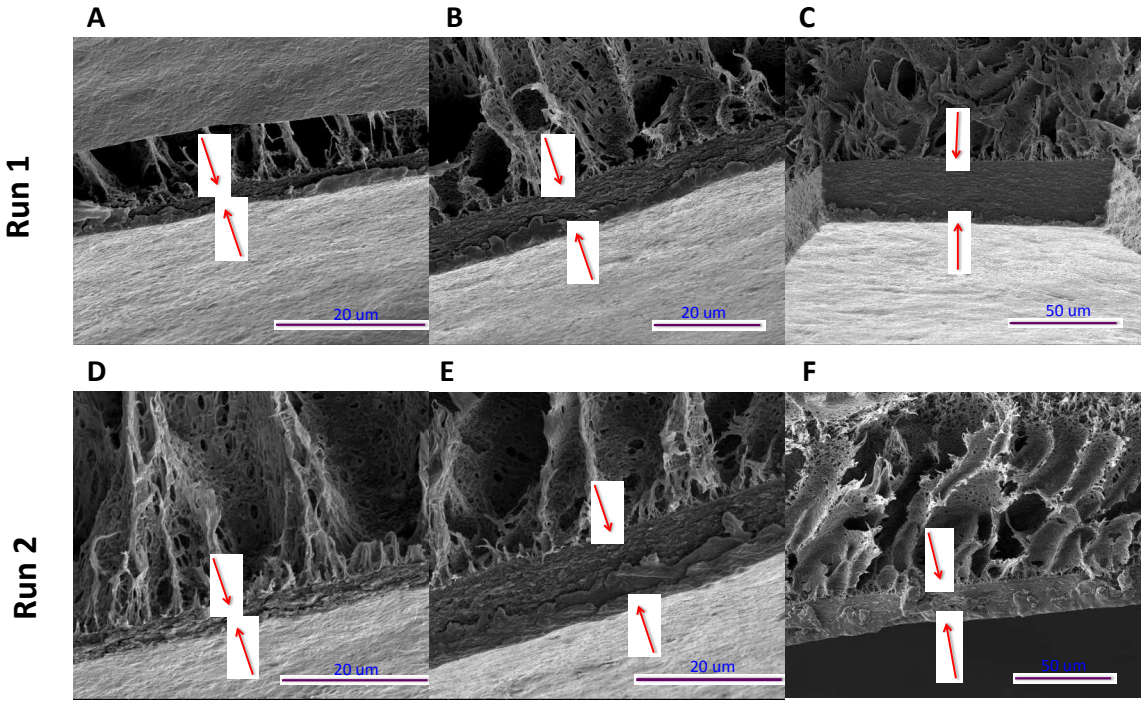

Figure S3. Cross-sectional images of membranes obtained by scanning electron microscopy. **(A)** F1, **(B)** F2 and **(C)** F3 membranes in Run 1; **(D)** F1, **(E)** F2 and **(F)** F3 membranes in Run 2.

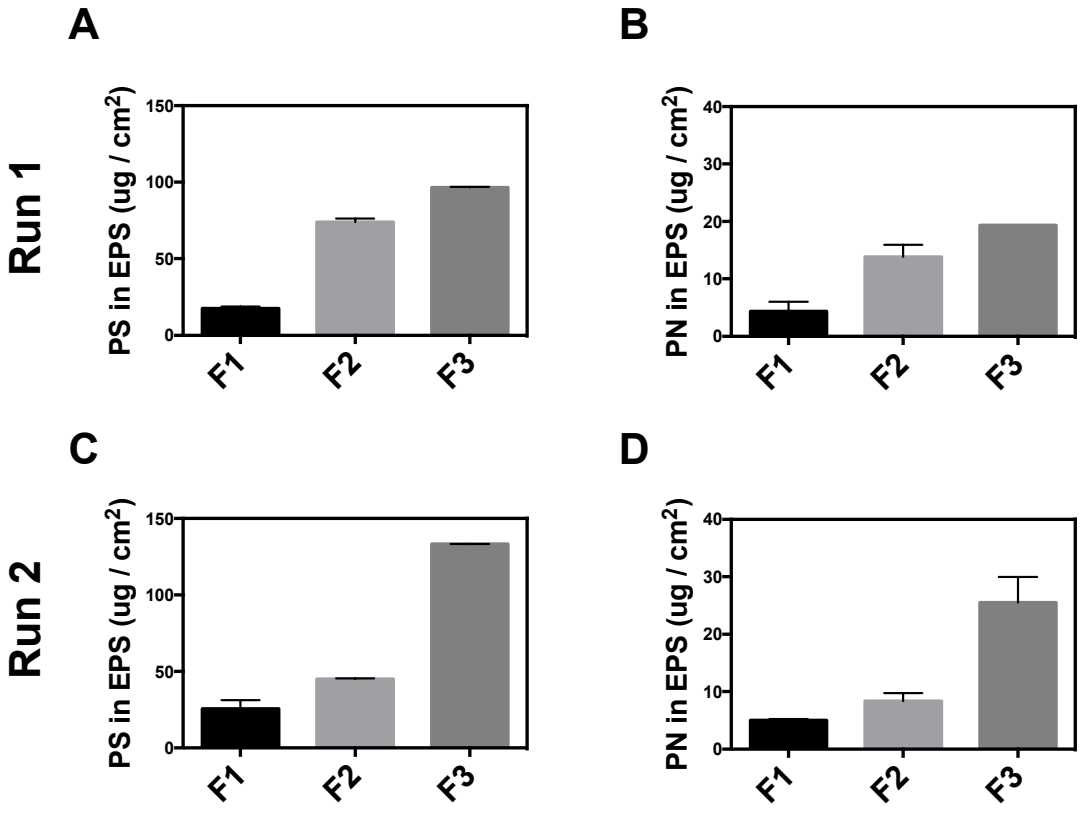

Figure S4. Concentration of polysaccharide (PS) and protein (PN) in soluble extracellular polymeric substance (EPS) attached on F1, F2 and F3 membranes. Fouling severity increase in the order of F1 through F3. **(A)** PS in Run 1; **(B)** PN in Run 1; **(C)** PS in Run 2; **(D)** PN in Run 2. Vertical bars indicate standard deviations obtained from measurements of three technical replicates.

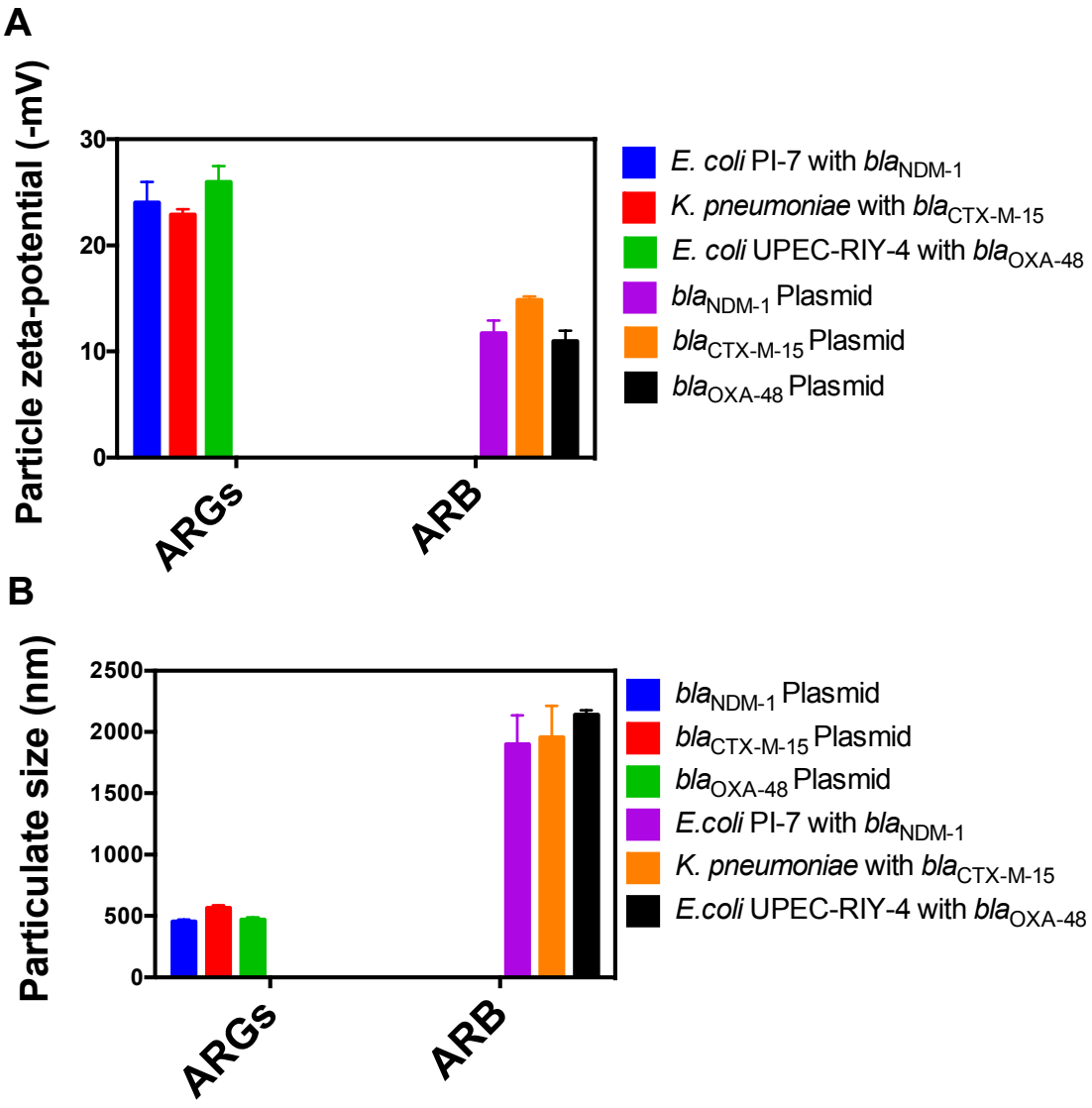

Figure S5. Physico-chemical properties of ARGs and ARB. **(A)** Particle sizes of ARGs and ARB evaluated by dynamic light scattering (DLS), and **(B)** zeta-potential of ARGs and ARB quantified by laser doppler velocimetry (LDV). Vertical bars indicate standard deviations obtained from measurements of three technical replicates.

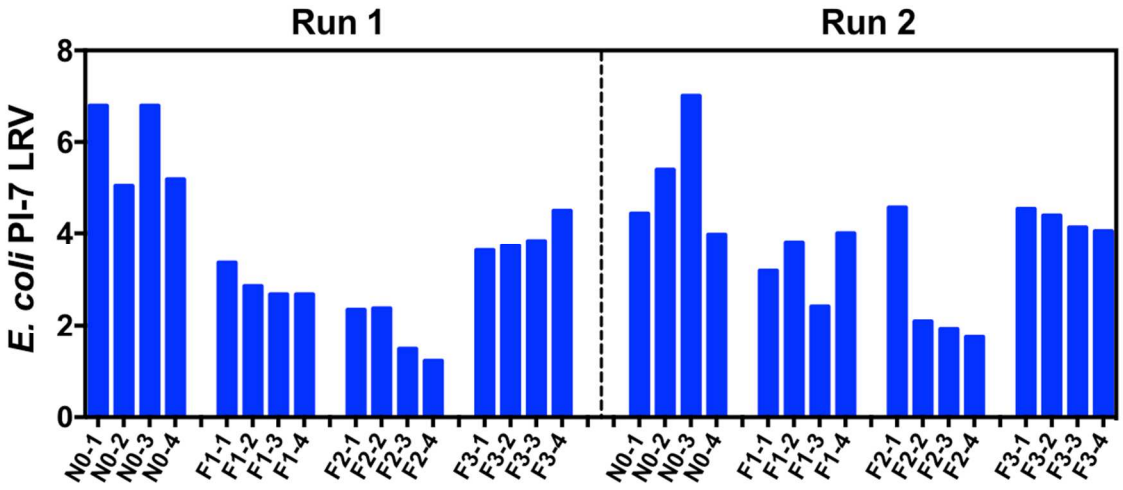

Figure S6. Log removal value (LRV) of *E. coli* PI-7 achieved by different membranes in both Run 1 and Run 2. LRV were evaluated by flow cytometry.

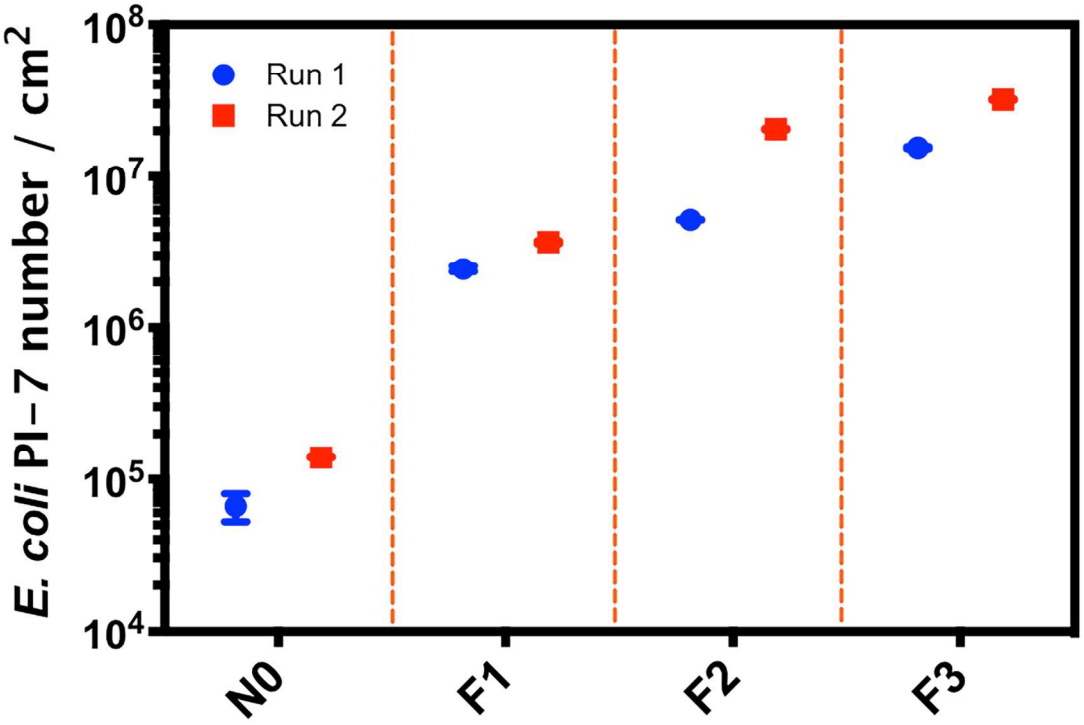

Figure S7. Abundance of *E. coli* PI-7 attached on different membranes in both Run 1 and Run 2. Abundances were quantified by flow cytometry. Vertical bars indicate standard deviations obtained from measurements of three technical replicates.

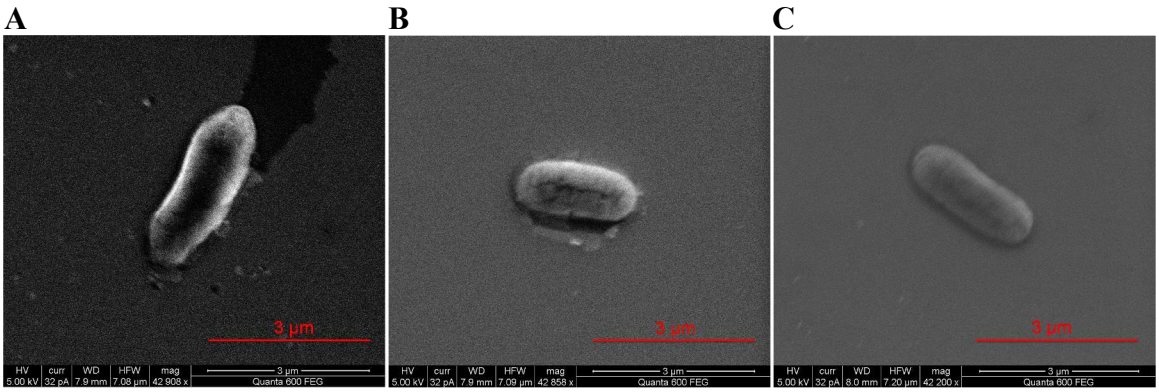

Figure S8. SEM images of ARB used in this study. **(A)** *E. coli* PI-7, **(B)** *K. pneumoniae* L7 and **(C)** *E.coli* UPEC-RIY-4.

References

1. Toh, B. E.; Bokhari, O.; Kutbi, A.; Haroon, M. F.; Mantilla Calderon, D.; Zowawi, H.; Hong, P.-Y., Varying occurrence of extended-spectrum beta-lactamase bacteria among three produce types. *Journal of Food Safety* **2017**. doi: 10.1111/jfs.12373
2. Mantilla-Calderon, D.; Jumat, M. R.; Wang, T.; Ganesan, P.; Al-Jassim, N.; Hong, P.-Y., Isolation and characterization of NDM-positive Escherichia coli from municipal wastewater in Jeddah, Saudi Arabia. *Antimicrobial agents and chemotherapy* **2016,** *60*, (9), 5223-5231.
3. Harb, M.; Wei, C.-H.; Wang, N.; Amy, G.; Hong, P.-Y., Organic micropollutants in aerobic and anaerobic membrane bioreactors: Changes in microbial communities and gene expression. *Bioresource technology* **2016,** *218*, 882-891.