Supporting Information

Photocatalytic Reactive Ultrafiltration Membrane for Removal of Antibiotic Resistant Bacteria and Antibiotic Resistance Genes from Wastewater Effluent

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Methods

TGA analysis

TGA (SDT-Q600, TA Instruments Inc., USA) was carried out from 30 to 800 °C at a heating rate of 10 °C/min under nitrogen atmosphere.

Energy-Dispersive X-ray analysis

Elemental mapping of the TiO_2-modified PVDF membrane was performed with an energy-dispersive X-ray (EDS) attached to the SEM. All membrane samples were coated with a 10-nm platinum layer before EDS analysis.

The determination of TiO_2 release

To evaluate TiO_2 leaching during filtration, titanium concentration in the permeate was measured after 12, 24, and 48 h of dead-end UF experiments with the TiO_2-modified PVDF membrane (4.1 cm^2) using the ICP-MS.

Brunauer-Emmett-Teller (BET) and Barrett–Joyner–Halenda (BJH) tests

Nitrogen adsorption–desorption measurements were carried out using a gas sorptometer (SSA-4300, Builder, China) at 77 K. The surface area, pore volume, and pore size were determined based on the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods.

qPCR measurement

The specificities of the primer pairs were verified by the NCBI primer BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The ARGs and integrons were amplified by PCR with plasmid DNA extracted from the wastewater and each PCR product was examined by agarose gel electrophoresis and gene sequencing. The DNA of target gene was purified using Qiagen Gel Extraction Kit (Qiagen, Germany) and ligated into a pMD 19-T vector (Takara, Japan) and cloned into *E. coli* DH5α competent cell (Takara, Japan). The plasmid standards were isolated from the positive clones and then analyzed by gene sequencing to confirm the existence of the target genes. Thermocycling conditions were 95 °C for 30 s, followed by 40 cycles of 95 °C for 15 s, then the annealing temperature (corresponding to the gene sequence, designed by the

Oligo 7 software) for 30 s, and 72 °C for 30 s. A qPCR negative (nuclease-free water as template) was set in each qPCR batch.

LOD determination

Detection limit of 95% (LOD_{95}) for 20 replicates with positive amplification were applied for determining the detectable DNA concentration. The lowest concentration of positive target DNA was set as the initial LOD for a standard curve. The initial LOD was used as the base for 5-fold dilution series to determine the actual LOD. Probit Analysis was performed using the Statistical Package for Social Science software (SPSS) to calculate the LOD with a 95% probability.

Table S1. Primers used for the qPCR reaction in this work.

Target gene	Prime sequence (5'-3')	Product size (bp)	Ref.
floR	F: GAGGGTGTCGTCATCTACGG	138	[1]
	R: GAGCATCGCCAGTATAGCCA		
tetC	F: GCGGGATATCGTCCATTCCG	207	[2]
	R: GCGTAGAGGATCCACAGGACG		
tetW	F: GAGAGCCTGCTATATGCCAGC	168	[3]
	R: GGGCGTATCCACAATGTTAAC		
tetQ	F: AGAATCTGCTGTTTGCCAGTG	167	[4]
	R: CGGAGTGTCAATGATATTGCA		
sul1	F:CGCACCGGAAACATCGCTGCAC	162	[3]
	R: GAAGTTCCGCCGCAAGGCTCG		
sul2	F: TCCGGTGGAGGCCGGTATCTGG	185	[5]
	R: CGGGAATGCCATCTGCCTTGAG		
intI1	F: CCTCCCGCACGATGATC	280	[6]
	R: TCCACGCATCGTCAGGC		
intI2	F: GACGGCTACCCTCTGTTATCTC	195	[7]
	R: TGCTTTTCCCACCCTTACC		
intI3	F: GCCACCACTTGTTTGAGGA	138	[7]
	R: GGATGTCTGTGCCTGCTTG		
16S	F: GCGGTGGAGCATGTGGTTTA	181	[1]
	R: GATAAGGGTTGCGCTCGTTG		

Table S2. Annealing temperatures and sequences of ARGs and integrons.

Target gene	Annealing temperature (°C)	Sequence	GC%
floR	55	gagggtgtcgtcatctacggccttttcagttcgatgctggcgttcgtgcctgcgc tcggccctatcgccggaacattgatcggcgagttcttgggatggcaggcgat attcattactttggctatactggcgatgctc	56
tetC	58	gctgctactccccgttaagtgcctgaacttctggttgtacacgttgtgttacgcc gctggaatgggtagcttcttcgtctttttctccattgcgcccggactaatgatgg gcaggca	59
tetW	54	gcgggatatcgtccattccgacagcatcgccagtcactatggcgtgctgcta gcgctatatgcgttgatgcaatttctatgcgcacccgttctcggagcactgtcc gaccgctttggccgccgcccagtcctgctcgcttcgctacttggagccactat cgactacgcgatcatggcgaccacacccgtcctgtggatcctctacgc	54
tetQ	52	agaatctgctgtttgccagtggagcaacggaaaagtgcggccgtgtggata atggtgacaccataacggactctatggatatagagaaacgtagaggaatta ctgtccgggcttctacgacatctattatctggaatggagtgaaatgcaatatca ttgacactccg	46
sul1	62	cgcaccggaaacatcgctgcacgtgctgtcgaaccttcaaaagctgaagtc ggcgttggggcttccgctattggtctcggtgtcgcggaaatccttcttgggcgc caccgttggccttcctgtaaaggatctgggtccagcgagccttgcggcggaa cttca	59
sul2	59	tggaggccggtatctggcgccagacgcagccattgcgcaggcgcgtaagc tgatggccgaggggcagatgtgatcgacctcggtccggcatccagcaatc ccgacgccgcgcctgtttcgtccgacacagaaatcgcgcgtatcgcgccgg tgctggacgcgctcaaggcagatggcattcccg	66
intI1	59	tccacgcatcgtcaggcattggcggccttgctgttcttctacggcaaggtgctg tgcacggatctgccctggcttcaggagatcggaagacctcggccgtcgcgg	60

		cgcttgccggtggtgctgaccccggatgaagtggttcgcatcctcggttttctg gaaggcgagcatcgtttgttcgcccagcttctgtatggaacgggcatgcgga tcagtgagggtttgcaactgcgggtcaaggatctggatttcgatcacggcac gatcatcgtgcgggagg	
intI2	53	tgcttttcccacccttaccgtcatgcacagtgatgcagccattatcaaaatcaa aatctttaacccgcaaacgcaagcattcattaatgcgcaaacctgcaccata cagcagcgtaaaaataacttggttgcgagtatccataacctgcaaaatgcgt tgcacttcatttgcagagataacagagggtagccgtc	44
intI3	57	gccaccacttgtttgaggaaagactgaaccggcaactaaaaaaagcggta gttcaggctggcattgccaaacacgtatctgtccacaccctgcgccactcatt cgccacccacttgctgcaagcaggcacagacatcc	54

Table S3. Standard curves and LOD_{95} of ARGs and integrons detected by qPCR reaction.

Target gene	Efficiency	Mean square error	Slope	r^2	Detection Limit (Copies/mL)	Highest Cq measured
floR	1.948	0.0187	-3.453	0.9995	105.01	31.42
tetC	1.946	0.0199	-3.456	0.9971	849.87	29.85
tetW	1.982	0.044	-3.365	0.9961	1734.64	29.27
tetQ	1.987	0.0025	-3.352	0.9967	61.74	27.57
sul1	1.946	0.0030	-3.458	0.9999	68.82	29.84
sul2	1.975	0.0065	-3.384	0.9979	176.82	29.99
intI1	1.994	0.0013	-3.335	0.9927	467.73	32.31
intI2	1.959	0.0129	-3.425	0.9997	38.82	29.27
intI3	1.989	0.0025	-3.348	0.9994	85.41	31.54

Table S4. Spearman's correlation coefficients between degradation efficiency of ARGs and integrons in genome and plasmid and a proportion of guanine and cytosine to the total nucleotides sequences (GC%). Asterisks (*) indicate a significant correlation ($P < 0.05$).

Spearman's correlation coefficients	Location of ARGs and integrons	
	Genome	Plasmid
Pristine PVDF	0.908*	0.734*
TiO_2-modified PVDF	0.715*	0.642

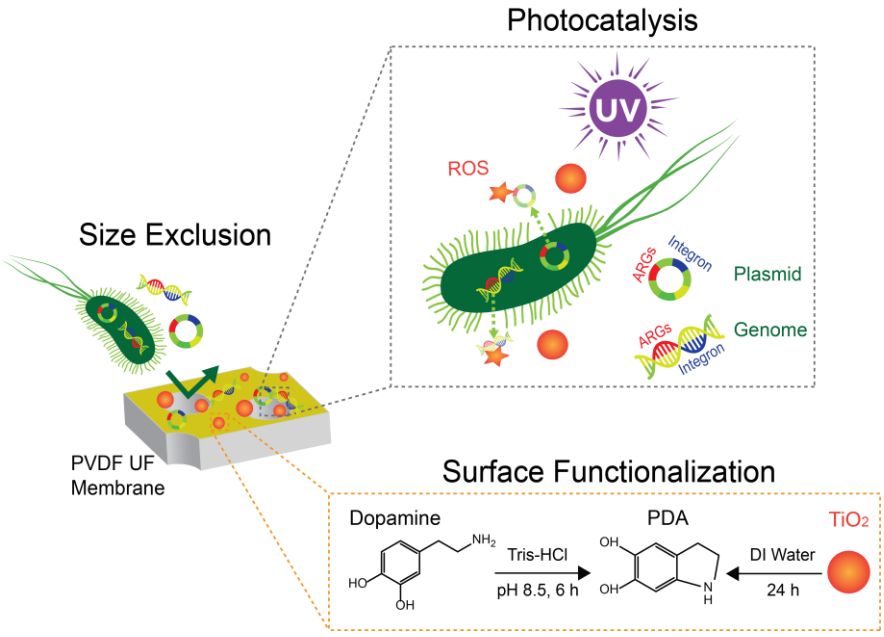

Figure S1. Scheme illustrating the functionalization of PVDF membrane with photocatalytic nanomaterials (TiO_2) and removal/degradation mechanisms of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) by the photocatalytic reactive UF membrane. The PVDF UF membrane is functionalized with TiO_2 nanoparticles, utilizing the catechol/quinone groups of polydopamine (PDA). Bacteria and ARB are retained by the TiO_2-modified UF membrane by size exclusion and subsequent UV treatment inactivates retained bacteria/ARB and degrades ARGs.

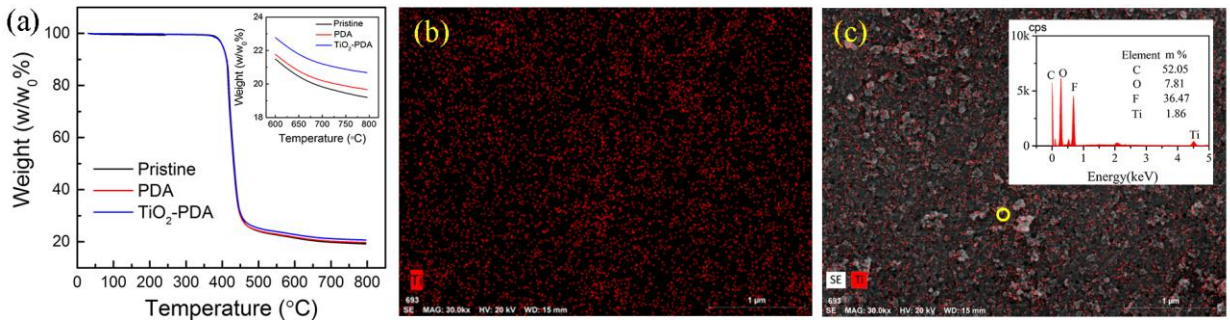

Figure S2. (a) Amount of TiO_2 incorporated in PVDF membranes measured via TGA. (b) Areal distribution of TiO_2 on the modified PVDF membrane surface analyzed by SEM-EDS. (c) Combined SEM and EDS elemental mapping images. The inset indicates EDS result from a single point scanning (indicated as yellow circle). TGA (SDT-Q600, TA Instruments Inc., USA) was carried out from 30 to 800 °C at a heating rate of 10 °C/min under nitrogen atmosphere.

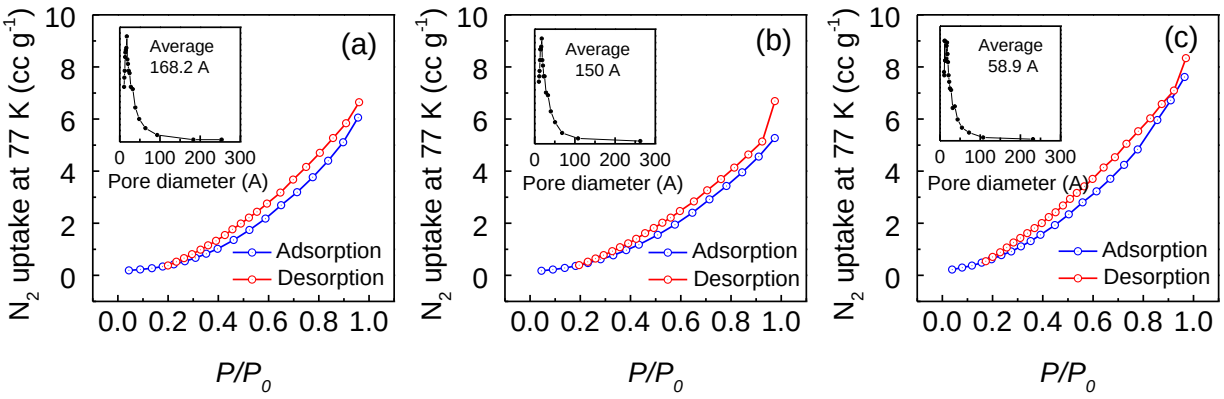

Figure S3. Nitrogen adsorption-desorption isotherms of (a) the pristine PVDF membrane, (b) PDA-coated PVDF membrane, and (c) TiO_2-modified PVDF membrane. The insets show membrane pore size distributions.

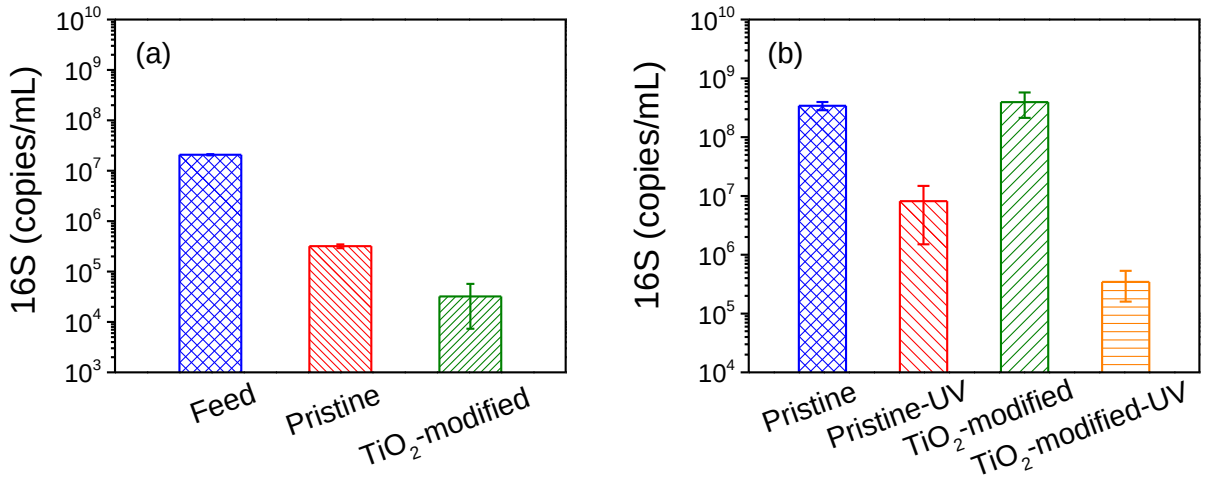

Figure S4. Abundance of 16S rRNA in (a) feed and permeate of pristine and TiO_2-modified PVDF membranes and (b) from bacteria retained on pristine and TiO_2-modified PVDF membranes before and after UV treatment. After filtration, wastewater effluent feed and permeate samples (40 mL) were centrifuged at 10,000 rpm for 20 min, and then the genome was extracted. For genome extraction from bacteria, membrane coupons with or without UV treatment were immersed in 5 mL sterile PBS and ultra-sonicated for 20 min. Immediately after, a 2 mL PBS solution was used to extract the genome from bacteria.

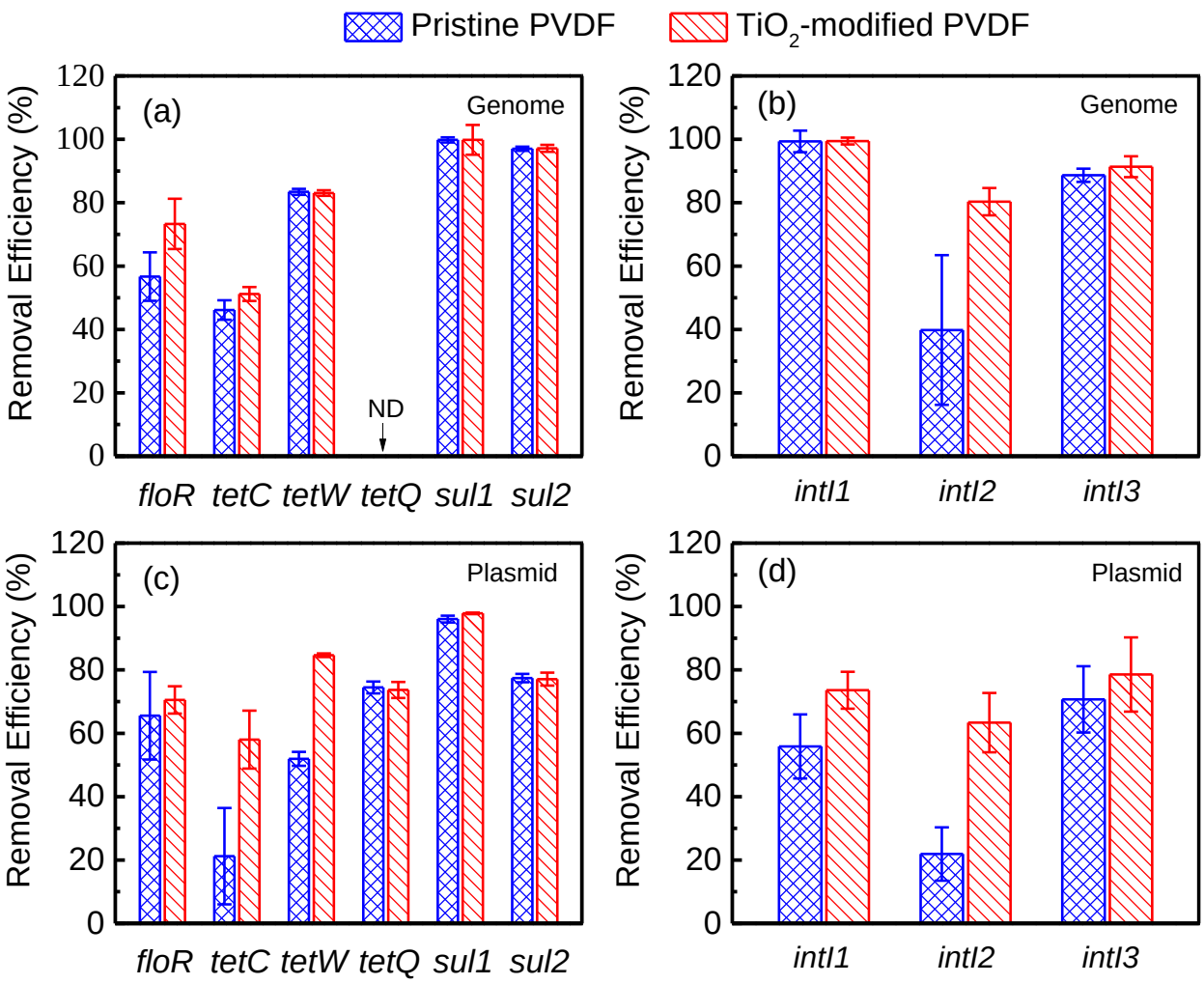

Figure S5. Removal efficiency of ARGs and integrons located in genome (a, b) and plasmid (c, d) by the pristine and TiO_2-modified PVDF membranes evaluated in UF experiments.

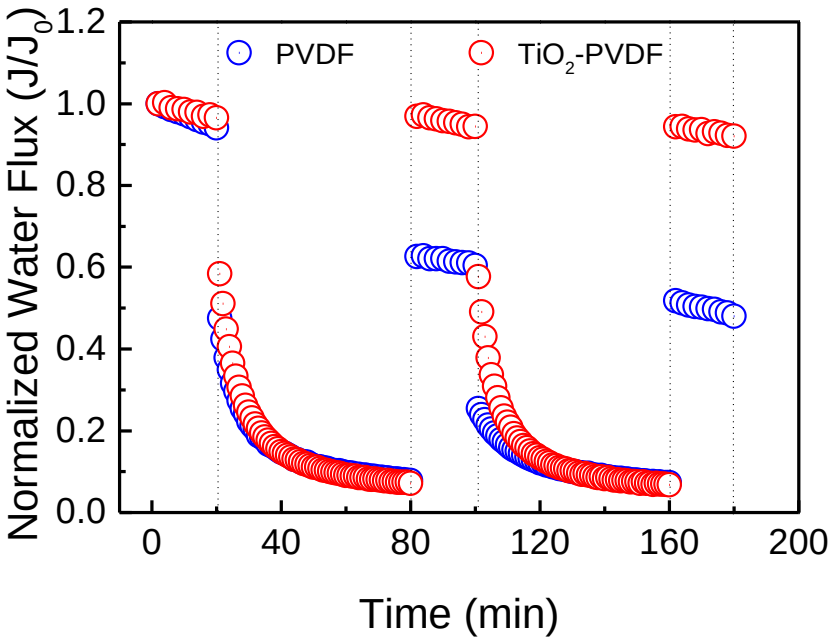

Figure S6. Normalized water flux for pristine PVDF membrane and TiO_2-modified PVDF membrane obtained from UF fouling experiments with wastewater effluent. Membranes were compacted for 3 h at 1.4 bar (20 psi) before UF experiments. Each fouling cycle comprised filtration of DI water for 20 min at applied pressure of 1.4 bar (20 psi) and secondary wastewater effluent for 60 min, followed by UV irradiation for 60 min.

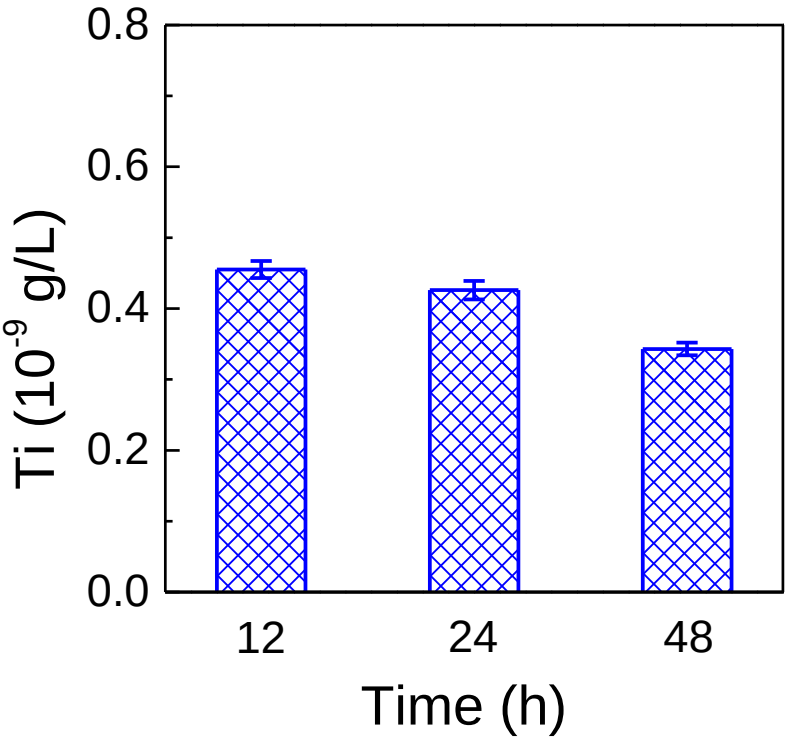

Figure S7. Amount of Ti leached to the permeate after UF experiments for 12, 24, and 48 h.

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