

## **Cytotoxicity of TiO<sub>2</sub> Nanoparticles toward *Escherichia coli* in Aquatic Environment: Effects of Nanoparticle Structural Oxygen Deficiency and Aqueous Salinity**

Yu-kun Qin,<sup>a,§</sup> Xing-pan Guo,<sup>a,§</sup> Fei-yun Tou,<sup>a</sup> Hui Pan,<sup>a</sup> Jing-nan Feng,<sup>a</sup> Jie Xu,<sup>b</sup> Bo Chen,<sup>c</sup> Min Liu,<sup>a</sup> Yi Yang,<sup>a,d,e\*</sup>

<sup>a</sup> Key Laboratory of Geographic Information Science (Ministry of Education); School of Geographical Sciences, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China

<sup>b</sup> Department of Geological Sciences, University of Texas at El Paso, 500 W University Avenue, El Paso, TX 79968, USA

<sup>c</sup> Center for Energy Harvesting Materials and Systems, 310 Durham Hall, Virginia Tech, Blacksburg, VA 24061, United States

<sup>d</sup> State Key Laboratory of Estuarine and Coastal Research, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China

<sup>e</sup> Shanghai Key lab for Urban Ecological Processes and Eco-Restoration, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China

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## 1. Growth curve of *E. coli*

We used spectrophotometer (722s) to measure the turbidity of *E. coli* in different time intervals so as to acquire the growth curve of *E. coli*. There were lag period (0-3h), logarithmic increased period (3-7h) and stable period (7-10h) for the growth of *E. coli*. The time of mid-log growth phase in our further experiments was chosen at 4h.

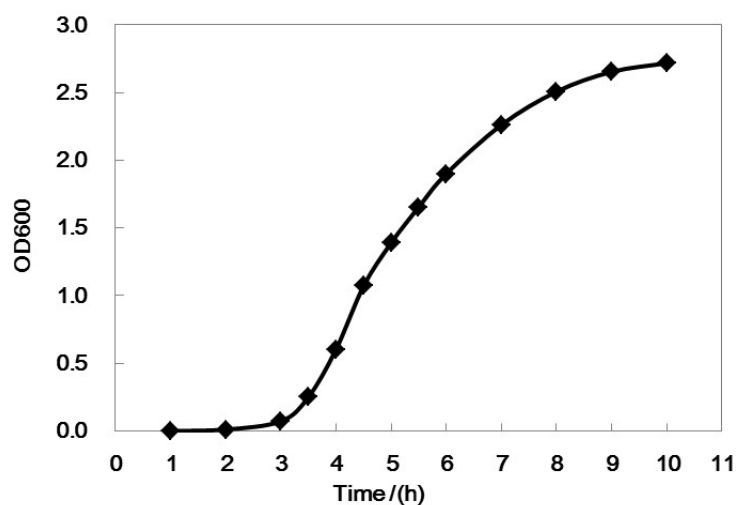


Fig. S1 The growth curve of *E. coli*

## 2. Properties of water samples taken along the Yangtze Estuary

Tab. S1 Physicochemical properties of three estuary samples

	Conductivity ( $\mu\text{s}/\text{cm}$ )	Salinity (‰)	DO (mg/L)	pH	DOC (mg/L)
QYK	3234	0.15	10.20	7.89	2.97
CY	4287	2.89	10.88	8.03	4.95
DH	8180	6.20	8.64	7.98	1.93

### 3. Toxicity kinetics-experiment of P25 to *E. coli*

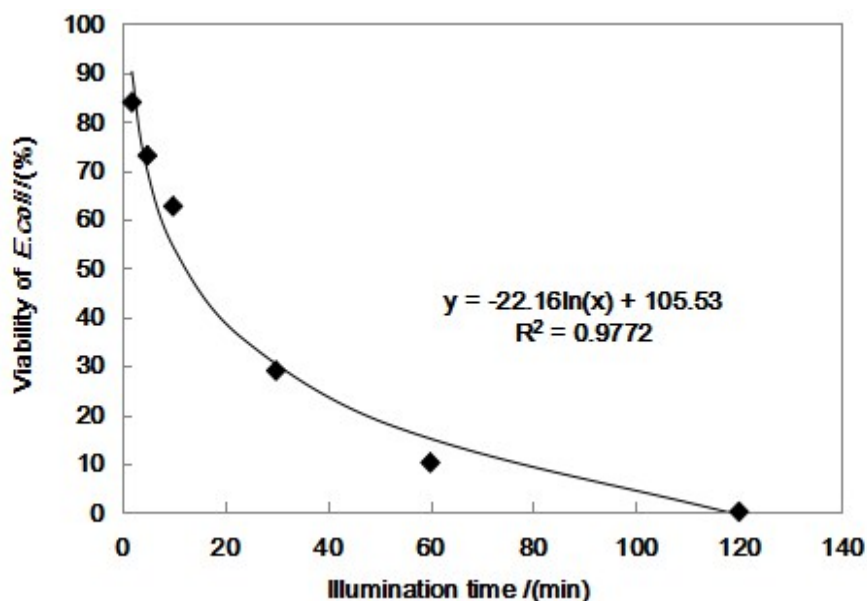


Fig. S2 Toxicity kinetics-experiment of P25 (100 mg/L) to *E. coli* under the illumination of UVA-365nm with the time intervals of 0, 2, 5, 10, 30, 60, 120 min

### 4. Change of pH, hydrodynamic diameter and zeta-potential of six TiO<sub>2</sub>-NPs in SDW

Tab. S2 pH, hydrodynamic diameter and Z-potential in SDW before and after the irradiation of UVA at different concentration of P25, P25-OD, An, An-OD, Ru and Ru-OD

	Concentration/ (mg/L)	Before irradiation			After irradiation		
		pH	Size/(nm)	Potential/(mv)	pH	Size/(nm)	Potential/(mv)
<b>P25</b>	10	6.69	218.87	20.30	6.32	227.33	16.57
	50	6.62	223.97	24.10	6.12	226.33	19.93
	100	6.68	240.17	27.47	6.00	239.33	22.57
	200	6.70	214.57	29.70	5.98	218.50	19.67
<b>P25-OD</b>	10	6.52	380.80	11.90	6.40	704.13	6.23
	50	6.43	210.53	20.03	6.02	220.07	14.53
	100	6.45	218.27	22.17	5.87	241.60	17.97
	200	6.37	202.57	25.47	5.98	215.30	19.50
<b>An</b>	10	6.12	579.27	12.80	6.07	761.27	5.74
	50	6.40	458.73	12.73	6.19	559.27	6.92
	100	6.23	454.30	13.13	6.08	468.23	10.83
	200	6.23	360.60	13.07	6.06	394.97	12.37

<b>An-OD</b>	10	6.36	318.10	-24.47	6.35	311.40	-19.37
	50	6.53	309.03	-30.33	6.31	304.07	-23.40
	100	6.43	217.50	-31.77	5.97	210.10	-27.10
	200	6.53	48.40	-32.40	5.81	47.42	-29.30
<b>Ru</b>	10	6.27	312.23	5.04	6.31	322.53	8.08
	50	6.21	241.70	17.27	6.21	407.73	6.26
	100	6.19	233.10	15.17	6.18	504.60	6.41
	200	6.09	176.17	21.10	6.09	282.53	1.98
<b>Ru-OD</b>	10	6.25	422.87	0.55	6.30	645.77	2.92
	50	6.22	354.43	6.91	6.25	783.57	3.60
	100	6.13	346.53	13.77	6.08	571.90	5.84
	200	5.97	155.67	18.60	5.98	244.90	12.47

HD: Hydrodynamic Diameter

Tab. S3 pH variation of different aqueous solutions with the addition of P25 and P25-OD

	SDW	1‰	5‰	10‰	30‰	QYK	CY	DH
Original pH	6.76	6.51	6.52	7.24	7.71	8.12	8.01	7.88
P25	10 mg/L	6.69	6.21	6.43	6.66	7.69	8.09	7.86
	50 mg/L	6.61	6.13	6.39	6.39	7.59	8.04	7.84
	100 mg/L	6.68	6.12	6.37	6.23	7.47	7.97	7.80
	200 mg/L	6.70	6.32	6.24	6.16	7.16	7.82	7.79
P25-OD	10 mg/L	6.52	6.24	6.32	6.87	7.54	8.11	8.04
	50 mg/L	6.43	6.31	6.32	6.72	7.43	8.08	8.01
	100 mg/L	6.47	6.65	6.27	6.60	7.26	8.05	7.96
	200 mg/L	6.37	6.90	6.22	6.41	6.95	7.97	7.93

## 5. Design of antioxidant genes primer

Primers of antioxidant and osmoregulatory genes were designed based on the target gene sequences from the whole genome sequence of *E. coli* (ATCC 25922).

Tab. S4: The cDNA sequences

Primer	Sequence (5'-3')
OxyR-F	TACCGTACTGCGTGAGGTGA
OxyR-R	GTAAGTGGTGGGTCTGTGCTT

SodA-F	CCTGCCAGAATTTGCCAACC
SodA-R	GCGTGACCACCAGCGTTATT
SodB-F	CGAATTACCTGCACTACCAT
SodB-R	GTTGTTGAATACGCCACCTT
AhpC-F	CCACAAAGCATGGCACAGCA
AhpC-R	CAACGAAGGTCGCACGGTCA
AhpF-F	GAAAGGCGACGGTAGCAAAG
AhpF-R	CAGCAGACCAATCTGGACGA
KatE-F	GGAAGTGACTGCGGATGACG
KatE-R	ATCGGTTTAAGGTGTTTGTAGG
16S rRNA-F	CCTACGGGAGGCAGCAG
16S rRNA-R	ATTACCGCGGCTGCTGG
OmpR-F	AAGATTCTGGTGGTTGATGACG
OmpR-R	AGGCGATCCATCTGTTCTGC
OmpC-F	TGTCGGCGGATCTATCACTT
OmpC-R	GATGTTGTTAGCGTCGTATTT
OmpF-F	ACAAAGCAACCCTGAAACCG
OmpF-R	CAGGTAATCAACAACGGACT

\*F, forward; R, reverse.

## 6. TEM and XRD of TiO<sub>2</sub> (P25, anatase and rutile)

TEM was applied to determine the primary size and morphology of Ti oxides NPs. Anatase and rutile NPs are in general rod-like particles with primary size of 10-35 nm (B) and 20-60 nm (C), respectively. while P25 is cubic or spherical with primary size of 10-25 nm (A). Annealing of the TiO<sub>2</sub> NPs to generate structural oxygen vacancies did not significantly change their sizes.

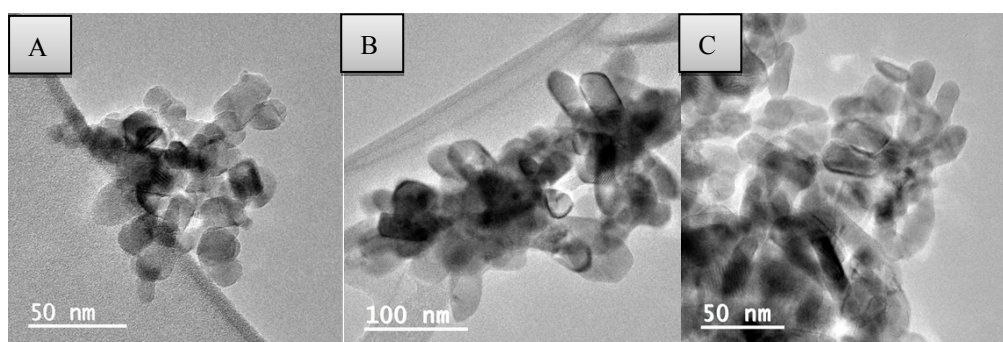


Fig. S3 TEM images for P25 (A), anatase (B) and rutile (C)

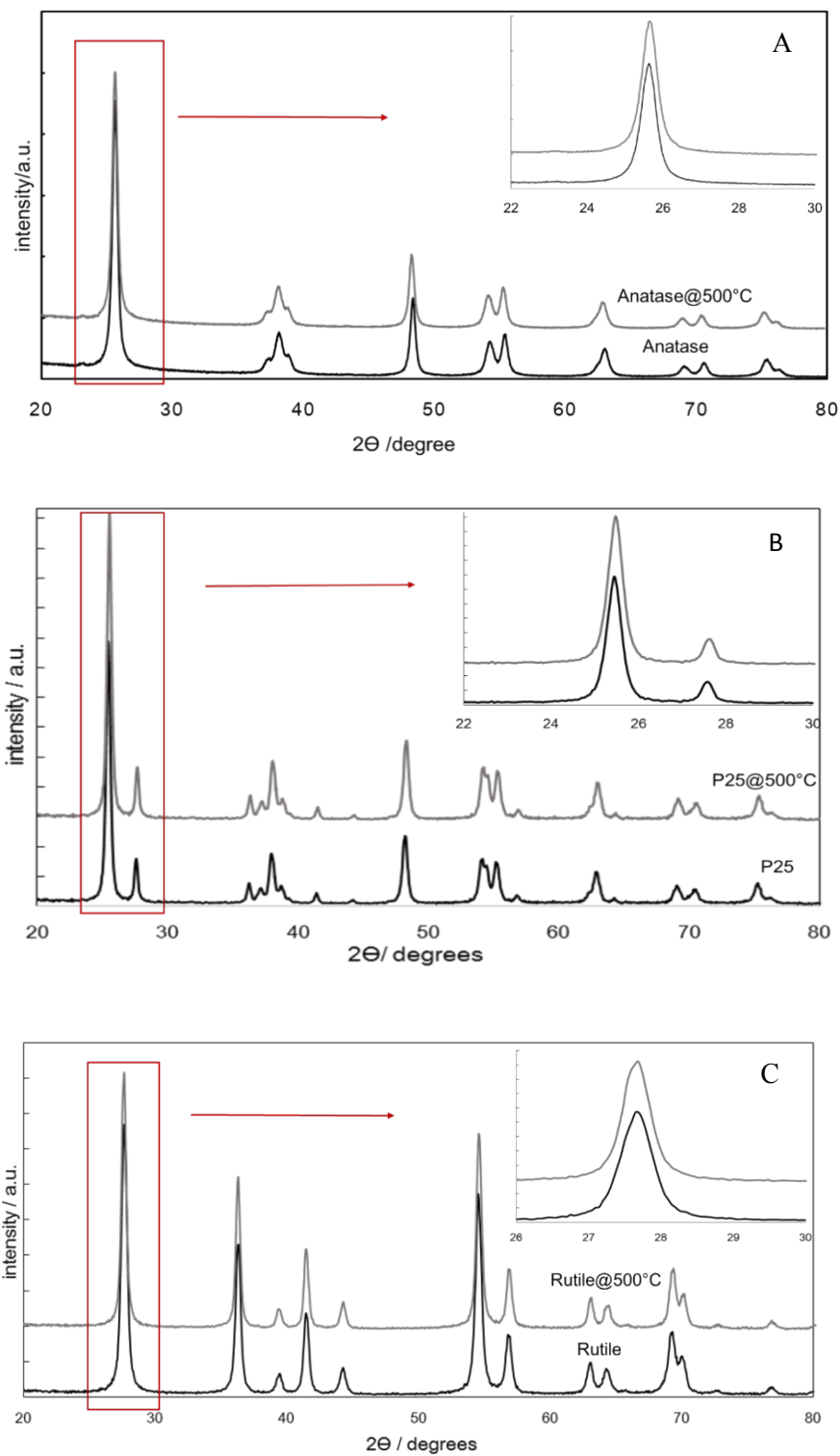


Fig. S4 Comparison of XRD patterns of anatase (A), P25 (B), and rutile (C) NPs before and after annealing at 500°C. The enlarged first dominant peaks showed the full width half maximum (FWHM) of XRD peak is similar, indicating the particle size didn't change too much after annealing.

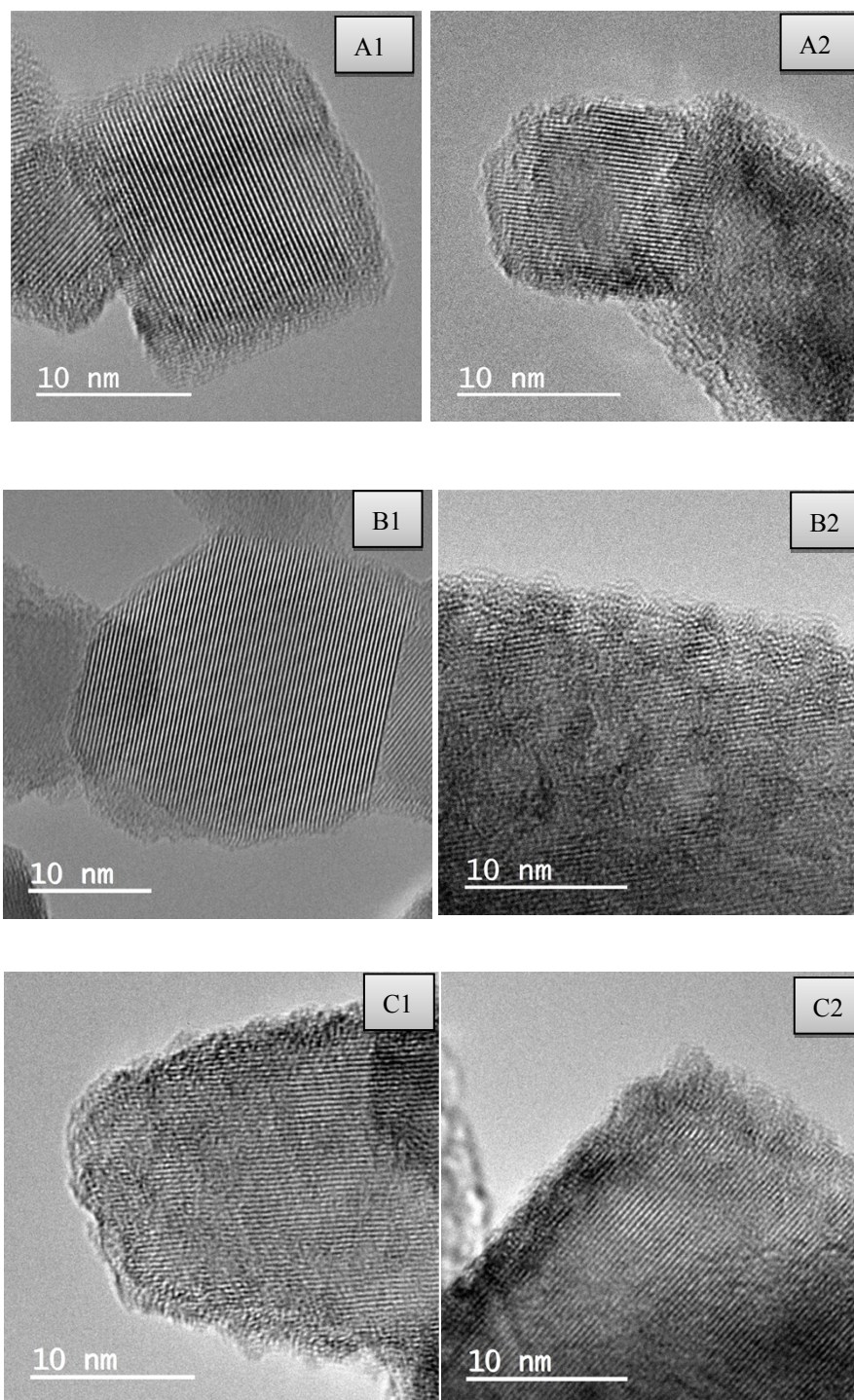


Fig. S5 HR-TEM images for P25-OD (A1-2), anatase-OD (B1-2), rutile-DO (C1-2)

## 7. Dose-response of *E. coli* to the six commercial TiO<sub>2</sub>-NPs in dark

All these TiO<sub>2</sub>-NPs and their oxygen deficient TiO<sub>2</sub> showed negligible toxicity to *E. coli* in dark. (Fig. S6)

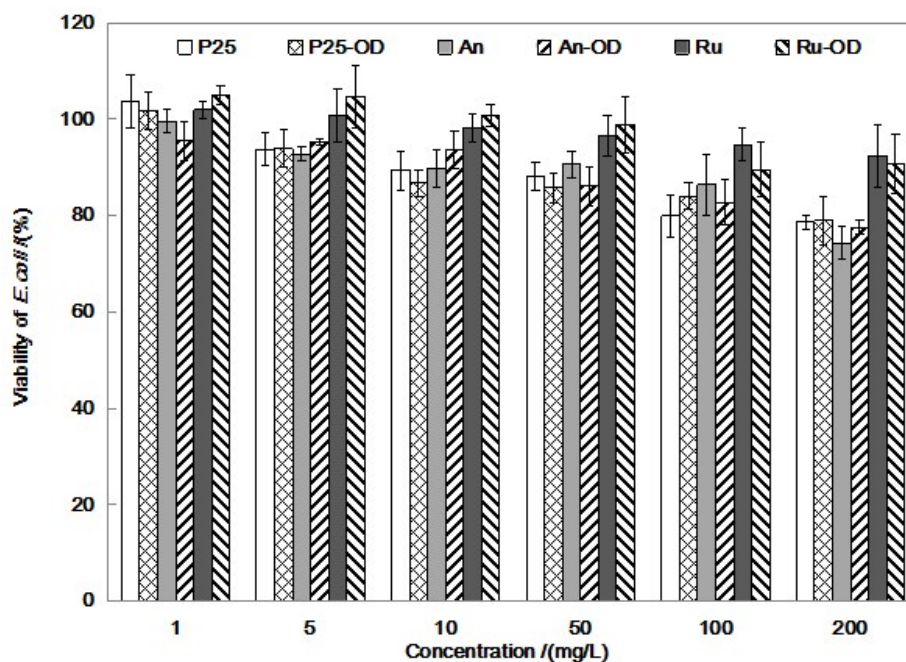


Fig. S6 Dose-response of *E. coli* to the six commercial  $\text{TiO}_2$ -NPs (suspended in sterile deionized water, SDW ) under dark condition for 30 min.

## 8. Photocatalytic degradation of methyl blue by six $\text{TiO}_2$ -NPs

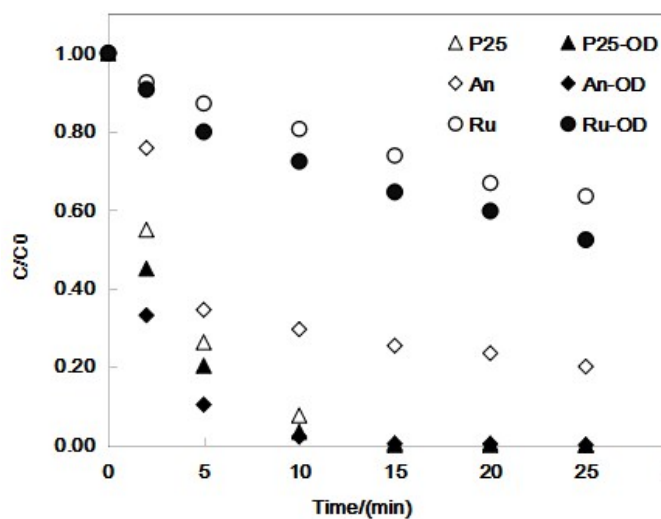


Fig. S7 Contradistinctive photocatalytic degradation of methyl blue (MB) by six kinds of  $\text{TiO}_2$ -NPs (100 mg/L) under different simulated solar irradiation time

## 9. Titanium ion release from six $\text{TiO}_2$ -NPs in water at different salinity, with and without UVA radiation



Tab. S5 Ti ion concentrations ( $\mu\text{g/L}$ ) in water at different salinity, with (A) and without (B) 30min UV irradiation at a  $\text{TiO}_2$  concentration of 100 mg/L

A	SDW	1‰	10‰
P25	1.13	0.42	< LOD
P25-OD	0.82	0.39	0.02
An	0.21	0.28	0.02
An-OD	0.18	0.21	< LOD
Ru	0.14	0.13	0.18
Ru-OD	0.45	0.23	< LOD

B	SDW	1‰	10‰
P25	0.45	0.54	0.03
P25-OD	1.20	0.36	0.08
An	0.48	0.06	0.10
An-OD	0.12	< LOD	< LOD
Ru	0.30	0.12	0.01
Ru-OD	0.41	0.32	< LOD

LOD: Limit of detection for Ti ( 0.01  $\mu\text{g/L}$ )

## 10. Effect of salinity on the cytotoxicity of *E. coli*

The effect of salinity on the cytotoxicity of *E. coli* was conducted in SDW with salinity gradient under UVA illumination shows no significant influence of salinity on the viability of *E. coli* ( $P > 0.05$ ).

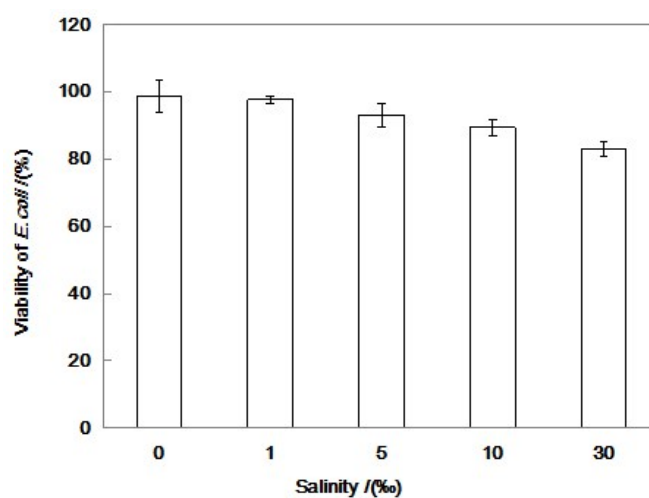


Fig. S8 Effect of salinity on the cytotoxicity of *E. coli* after 30 min-exposure under UVA irradiation

## 11. Hydrodynamic particle diameter and Z-potential variation of P25 and P25-OD at different salinity

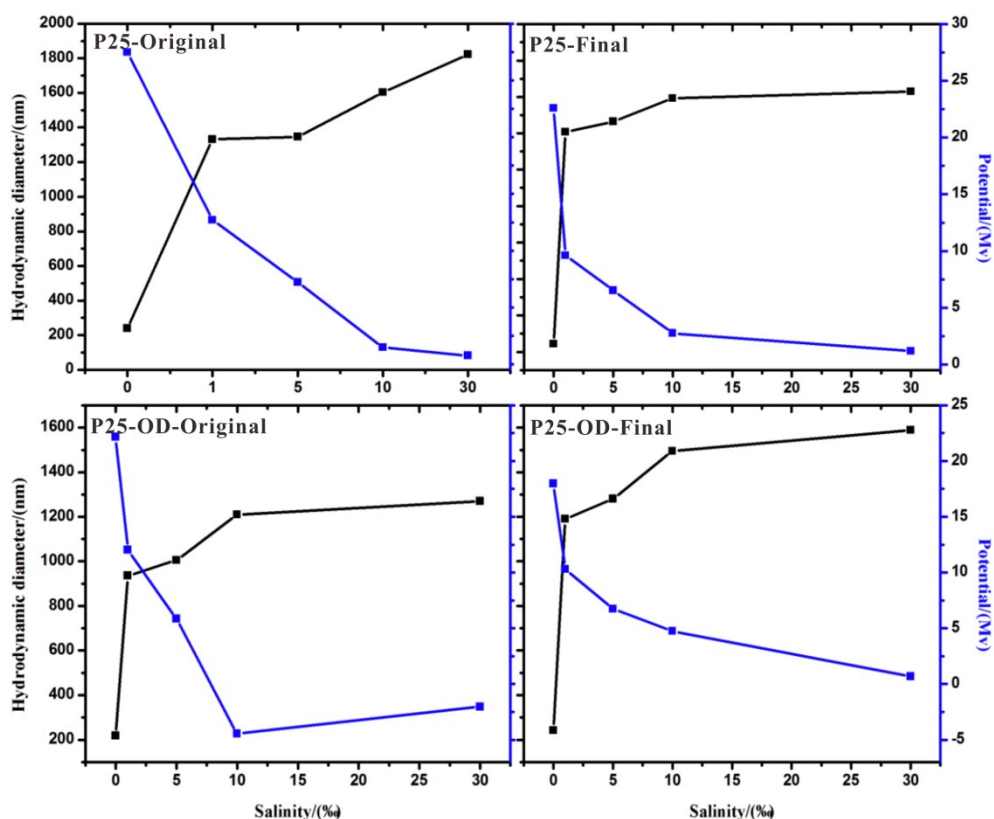


Fig. S9 Hydrodynamic diameter and Z-potential variation of P25 and P25-OD with different water salinity, before (the original) and after (the final) the irradiation of UVA for 30 min at the concentration of 100 mg/L

## 12. The relative expression of tested genes in *E. coli*

### 12.1 The relative expression of osmoregulatory genes

The osmoregulation is an important process for microbe to adapt to the salinity in the environment<sup>(1)</sup>. When the extracellular osmotic pressure increases, the EnvZ-OmpR system is activated in *E. coli*, and the gene OmpR will down-regulate the expression of the gene OmpF and up-regulate the expression of the gene OmpC simultaneously<sup>(2,3)</sup>. The outer membrane protein produced by the expression of the gene OmpC could accumulate osmoregulatory solutes such as K<sup>+</sup>, amino acids, and polyols from extracellular environment to improve the cytoplasmic levels<sup>(4)</sup>. However, all three genes showed down-regulated with increasing salinity (Fig. S10), which was

an abnormal regulation for *E.coli*, and this may indicate the inhibited activities of bacteria in brackish water without osmoregulatory solutes in present study. Previous studies also proved that the activation of *E.coli* was suppressed in NaCl solution at 24‰ or higher<sup>(5)</sup> and the high external concentration of NaCl would weak the ability of osmoregulation in non-halophilic *E.coli* so that their cellular activities such as division, transport and respiration are inhibited<sup>(6)</sup>. Therefore, we considered that under osmotic stress, although bacterial cells survived, the cellular activity including the osmoregulation ability had decreased in present study.

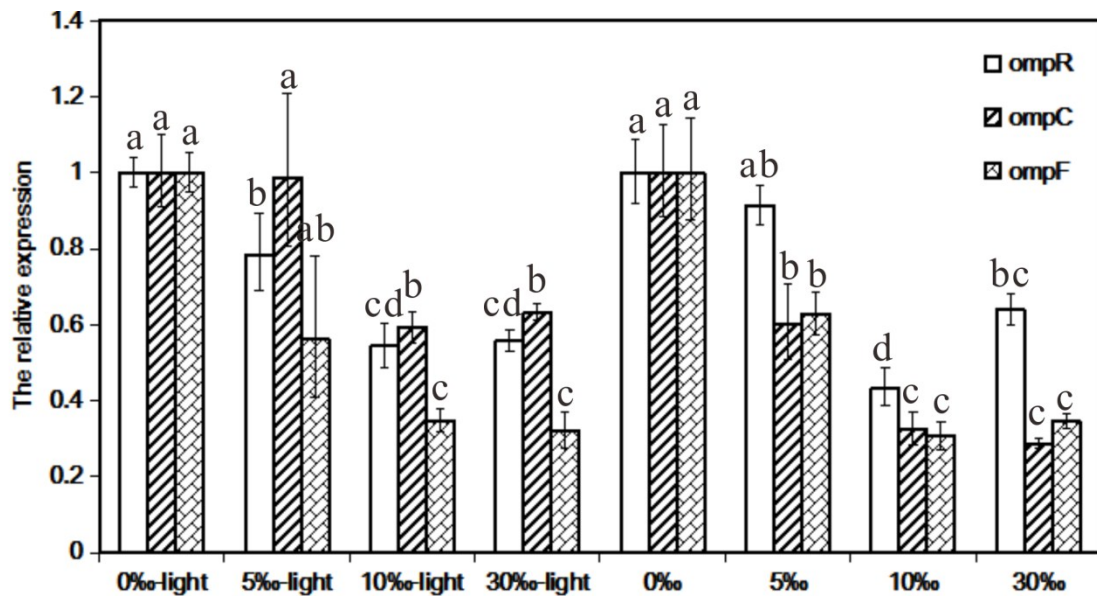


Fig. S10 The relative expression of three tested osmoregulatory genes under the treatment of different salinity with and without UVA irradiation. The letters above error bars indicate statistically significant differences between different salinity of the same gene ( $P < 0.05$ ) based on Tukey's HSD test.

### 12.2 The relative expression of antioxidant genes

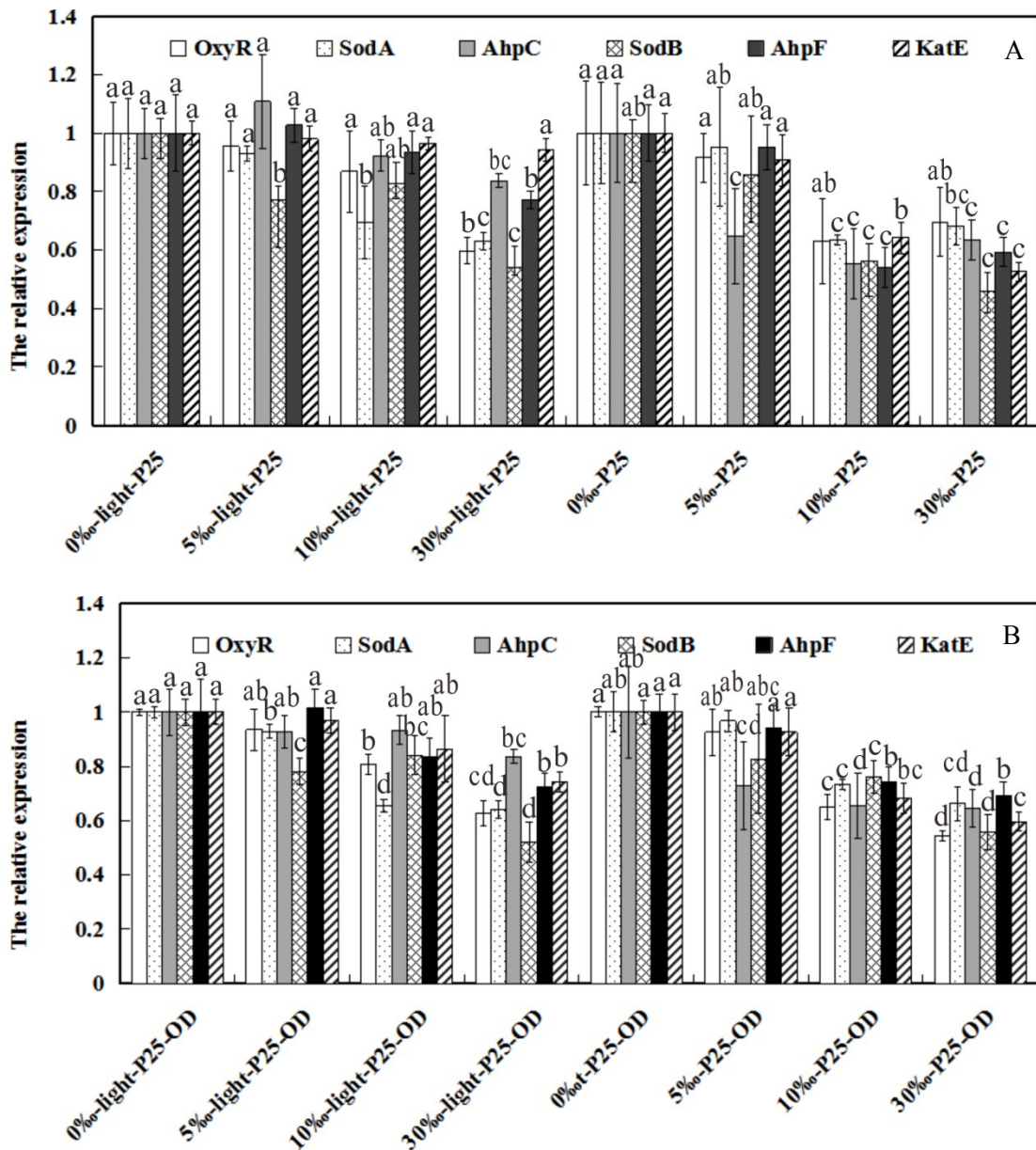


Fig. S11 The relative expression of six tested genes under the treatment of different salinity with and without UVA irradiation at the concentration of 100mg/L (A: P25; B: P25-OD). The letters above error bars indicate statistically significant differences between different salinity of the same gene ( $P < 0.05$ ) based on Tukey's HSD test.

### 13. Phototoxicity of P25 and P25-OD to *E. coli* in different estuarine water

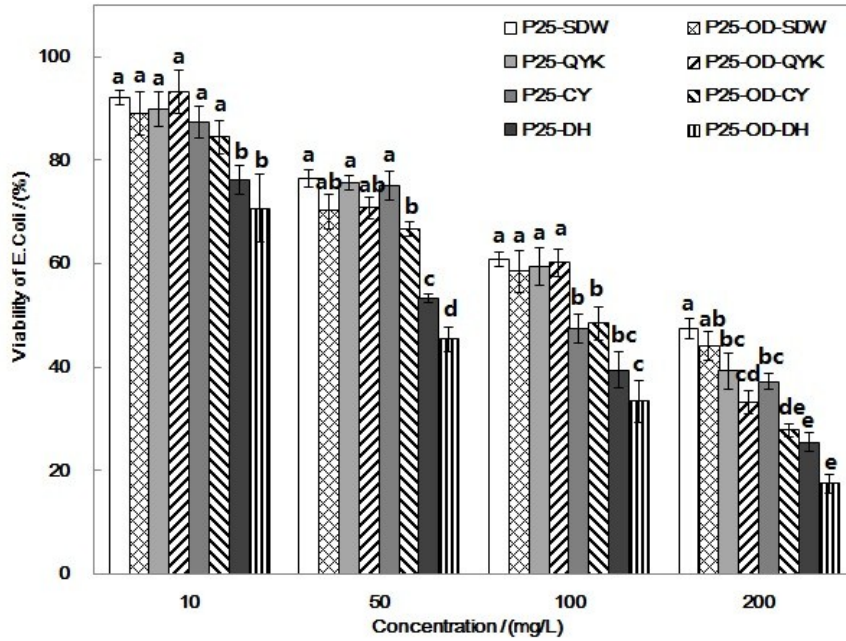
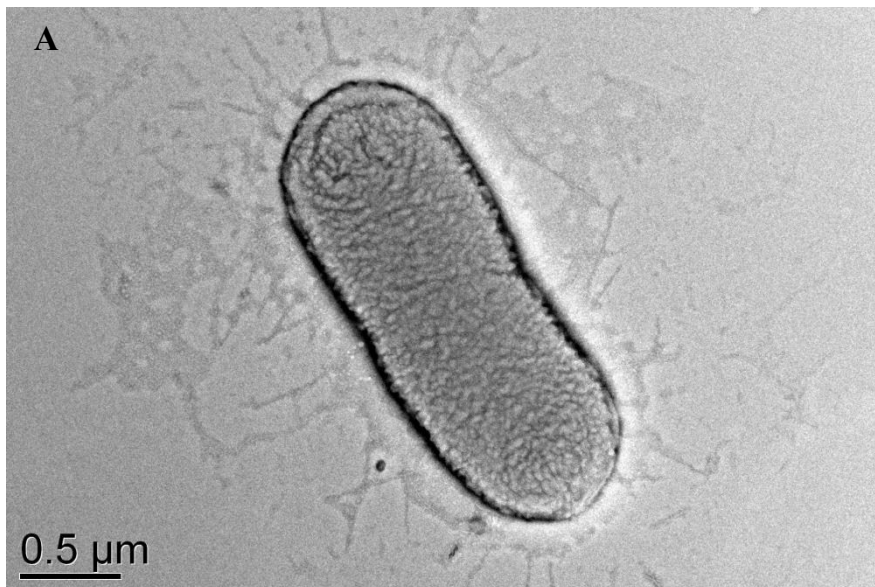


Fig. S12 Cytotoxicity of P25 and P25-OD to *E. coli* under UVA illumination for 30 min in different estuarine water compared to SDW. Different letters above the error bars indicate statistically significant differences between P25 in different estuarine waters ( $P < 0.05$ ) based on Tukey's HSD test.

#### 14. TEM images of *E. coli*, with/without exposure by P25 under UVA irradiation



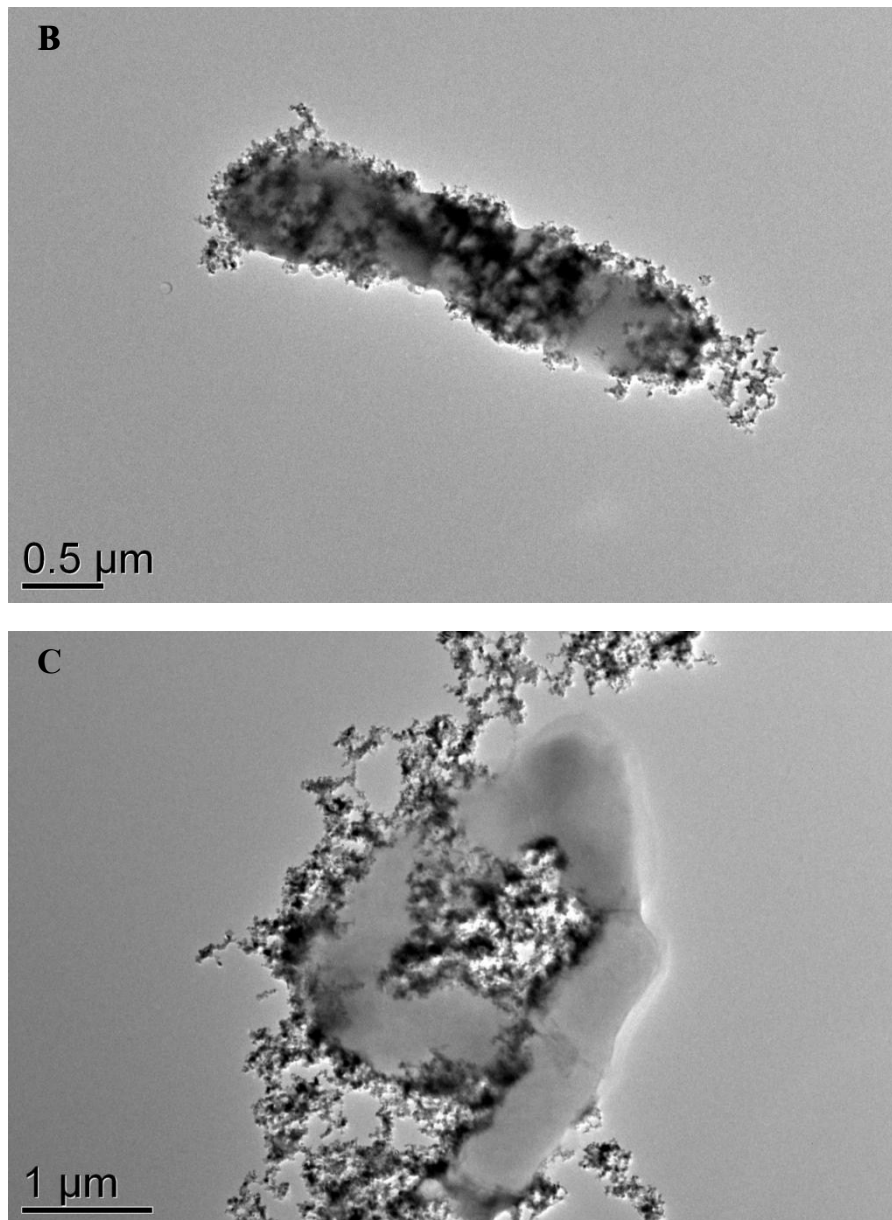


Fig. S13 TEM images of *E. coli* after irradiation by UVA for 30 min (A), P25 with *E. coli* after irradiation by UVA for 30 min (B) and *E. coli* with P25 at a salinity of 10‰ after irradiation by UVA for 30 min (C), showing that TiO<sub>2</sub> NPs can adsorb onto *E. coli*, and further cause the visible deformation of *E. coli* cell after irradiation by UVA for 30 min.

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- (2) L. A. Egger, H. Park, M. Inouye, Signal transduction via the histidyl-aspartyl phosphorelay, *Genes. Cells.*, 1997, **2**, 167-184.

- (3) H. Kondo, A. Nakagawa, J. Nishihira, Y. Nishimura, T. Mizuno and I. Tanaka, *Escherichia coli* positive regulator OmpR has a large loop structure at the putative RNA polymerase interaction site, *Nat. Struct. Biol.*, 1997, **4**, 28-31.
- (4) M. F. Roberts, Organic compatible solutes of halotolerant and halophilic microorganisms, *Aquat. Biosyst.*, 2005, **1**, 1-30.
- (5) K. Oguma, K. Izaki, H. Katayama, Effects of salinity on photoreactivation of *Escherichia coli* after UV disinfection, *J. Water. Health*, 2013, **11**, 457-464.
- (6) S. Nagata, Y. Maekawa, T. Ikeuchi, Y. B. Wang, and A. Ishida, Effect of compatible solutes on the respiratory activity and growth of *Escherichia coli* K-12 under NaCl stress, *J. Biosci. Bioeng.*, 2002, **94**, 384–389.