

Supporting Information

1 Materials and methods

1.1 Design of Q-PCR primers

Reference gene primer sequence

16s rRNA F341 CCTACGGGAGGCAGCAG

16s rRNA R518 ATTACCGCGGCTGCTGG

Target gene primer sequence

napA z3F CGCGAACAAGCTGATGAAGG

napA z3R AAGATCATCGGGATGTCGGC

nirS cd3aF G TSAACG TSAAGGARACSGG

nirS R3cd GASTTCGGRTGSGTCTTGA

norB Z1F CGTCGGTCAGATCCTCTTCG

norB Z1R GCGATGATCACGTAGAGCCA

nosZ 1527F CGCTGTTCHTCGACAGYCA

nosZ 1773R ATRTCGATCARCTGBTCGTT

sodB-Fn 5' ATCACTACGGCAAGCACCAT 3' 57.9

sodB-Rn 5' GAATACGCCACCTTCAGAGC 3' 57.3 119bp

1.2 Determination of LDH and SOD

The activity of LDH was determined using a lactate dehydrogenase assay kit (Jiancheng Bioengineering Institute Co. Ltd., Nanjing, China) according to the manufacturer's instructions. Finally, the absorbance was read at 440 nm using a TU1810 UV-vis spectrophotometer (Persee, Beijing, China). The strain precipitate was washed with 0.1 M PBS and centrifuged at 5000 r/min for 10 min twice prior to re-suspension in the same buffer at 4°C. Thereafter, the crude cell extract was prepared by ultrasound treatment (4°C, 20 kHz) for 5 min, after which it was freeze centrifuged at 12000 r/min for 10 min. The obtained supernatant was used for enzyme activity determination immediately. Intracellular SOD levels were measured using a Total Superoxide Dismutase (T-SOD) assay kit (Jiancheng Bioengineering Institute Co. Ltd., Nanjing, China). Finally, the absorbance was determined at 550 nm using a TU1810 UV-vis spectrophotometer

(Persee, Beijing, China). The concentration of the protein was determined by the Coomassie Brilliant Blue staining method, with bovine serum albumin as the standard. All tests in this study were conducted in triplicate.

1.3 The condition and calculation methods of qRT-PCR

The conditions of qRT-PCR was as follows: initial denaturation at 95°C for 3 min, followed by 45 cycles of denaturation at 95°C for 3 s and annealing and extension at 60°C for 30 s.

Relative fold changes of target genes were expressed in the form of $2^{-\Delta\Delta Ct}$. The efficiency of default primer amplification was 100%.

$\Delta Ct = Ct_{TAR} - Ct_{REF}$; Normalize Ct values for all TAR samples to the REF gene of its corresponding sample. Where Ct_{TAR} is the target gene of Ct value, Ct_{REF} is the reference gene of Ct value.

$\Delta\Delta Ct = \Delta Ct_{test} - \Delta Ct_{control}$; Normalize the TAR Mean ΔCt Expression to that of the Control to obtain $\Delta\Delta Ct$ Expression. Where ΔCt_{test} is the ΔCt value of *P. stutzeri* related genes (napA, nirS, cnorB and nosZ, respectively) in test sample. $\Delta Ct_{control}$ is the ΔCt value of *P. stutzeri* related genes (napA, nirS, cnorB and nosZ, respectively) in control sample.

Index conversion, finally, the results were expressed in the form of $2^{-\Delta\Delta Ct}$ to show the relative fold change of genes compared with the control group.

1.4 Effect of PFASs species on the denitrification process of the strain

To simply judge the influence of different PFASs on the denitrification process of the strain, we exposed the cultured strains to DM containing OBS, 6:2 FTS, N-EtFOSA, PFBS, PFH_xS, PFOS and PFOA, respectively. The final concentration of the pollutants in the culture medium was 200 mg/L. The strain was incubated at 150 r/min, 30°C for 20 h, and the supernatant was extracted by each 5 h to determine the nitrate concentration.

2 Figures and Tables

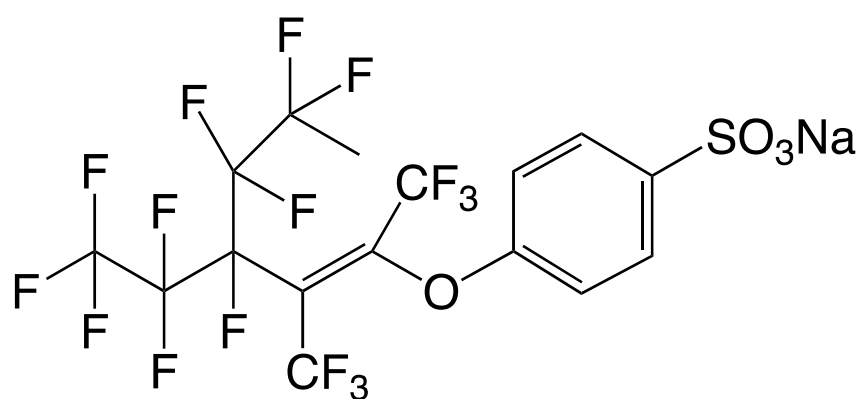


Fig. S1 OBS chemical structure

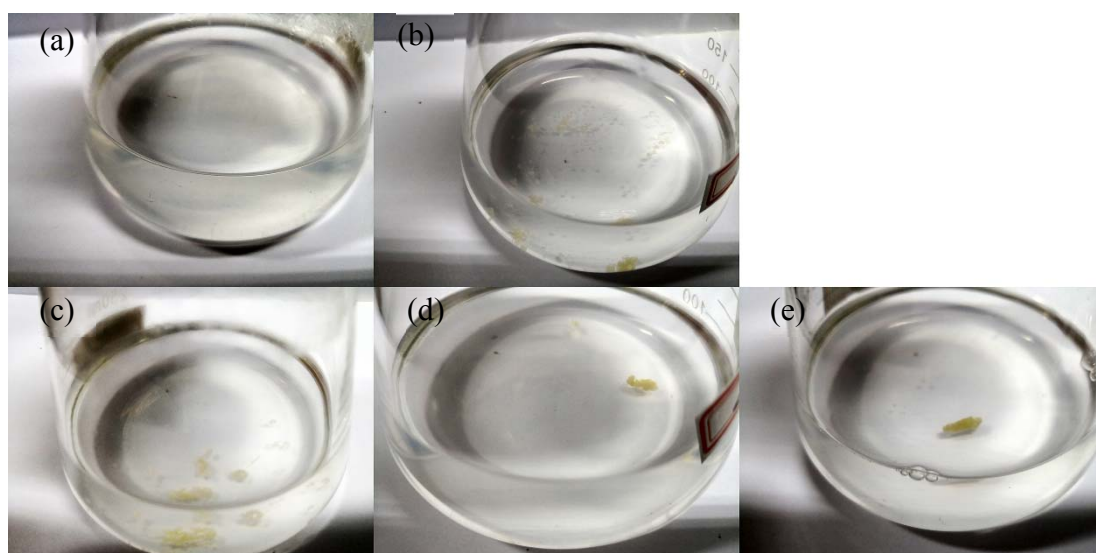


Fig. S2 Growth of *Pseudomonas stutzeri* strains cultured in DM for 5 h: (a) untreated group; (b–e) treated with 0.1 mg/L, 1 mg/L, 50 mg/L and 200 mg/L of OBS, respectively

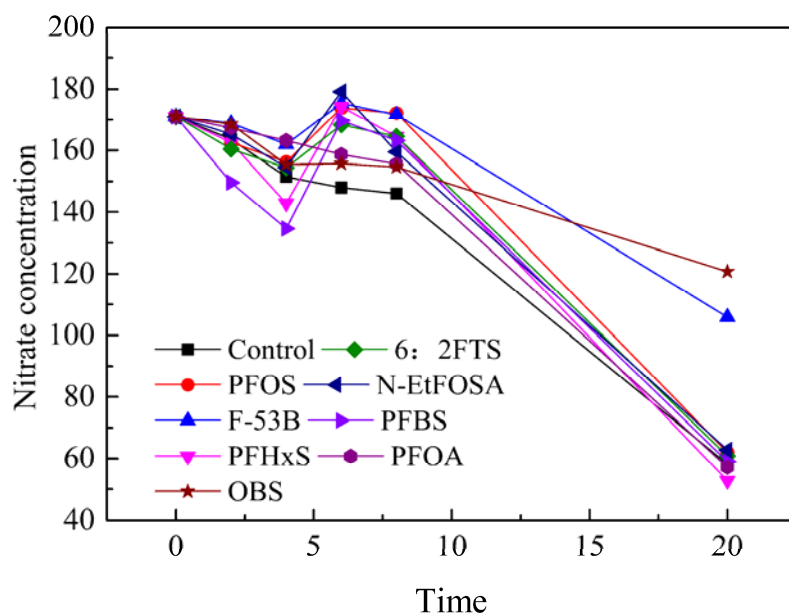


Fig. S3 Effect of different PFASs on the removal of nitrate by *P. stutzeri* [PFBS(K): $\text{CF}_3(\text{CF}_2)_3\text{SO}_3\text{K}$, PFH₆S(K): $\text{CF}_3(\text{CF}_2)_5\text{SO}_3\text{K}$, PFOS: $\text{CF}_3(\text{CF}_2)_7\text{SO}_3\text{K}$, 6:2FTS: $\text{CF}_3(\text{CF}_2)_5(\text{CH}_2)_2\text{SO}_3\text{K}$, F53B: $\text{C}_8\text{ClF}_{12}\text{H}_4\text{O}_4\text{SK}$, N-EtFOSA: N-ethyl perfluorooctane sulfonamide]

Table S1 Detailed information on the chemicals and materials used in this study

Chemicals and materials	Grade	Manufacturer
OBS	95%	Silworld Chemical Co. Ltd, Wuhan, China
Trizol extraction kit	–	Sangon Biotech Co. Ltd., Shanghai, China
SYBR Green PCR Master Mix	–	BBI Co. Ltd., Shanghai, China

Table S2 Significance analysis of OD_{600} change, where values with different letters (a–e) differ significantly ($p < 0.05$)

OBS concentration (mg/L)	Incubation time				
	5 h	0 h	15 h	20 h	25 h
0	b	b	b	c	bc
0.1	b	b	b	bc	abc
1	b	b	b	b	a
50	a	a	ab	c	c
200	a	a	a	a	ab

Table S3-a Significance analysis of nitrate removal, where values with different letters (a–e) differ significantly ($p < 0.05$)

OBS concentration (mg/L)	Incubation time					
	10 h	13 h	16 h	19 h	22 h	25 h
0	a	a	a	a	a	a
0.1	b	b	b	a	a	a
1	c	c	c	a	a	a
50	d	d	d	a	a	a
200	e	e	e	b	a	a

Table S3-b Significance analysis of nitrite removal, where values with different letters (a–e) differ significantly ($p < 0.05$)

OBS concentration (mg/L)	Incubation time					
	10 h	13 h	16 h	19 h	22 h	25 h
0	d	e	c	b	a	a
0.1	c	d	b	c	c	a
1	c	c	b	c	d	b
50	b	b	b	b	e	c
200	a	a	a	a	b	d

Table S3-c Significance analysis of ammonia nitrogen removal, where values with different letters (a–e) differ significantly ($p < 0.05$)

OBS concentration (mg/L)	Incubation time				
	5 h	10 h	15 h	20 h	25 h
0	a	a	ab	a	b
0.1	a	a	bc	a	c
1	a	a	c	b	e
50	a	a	a	a	a
200	a	b	d	c	d

Table S3-d Significance analysis of total nitrogen removal, where values with different letters (a–e) differ significantly ($p < 0.05$)

OBS concentration (mg/L)	Incubation time				
	5 h	10 h	15 h	20 h	25 h
0	b	ab	a	a	a
0.1	b	ab	ab	b	b
1	a	a	b	b	b
50	b	a	b	bc	b
200	b	b	c	c	c