Supplementary Material

Exploring on effects of unmodified and amine-functionalized polystyrene nanoplastics on nitrogen removal by *Pseudomonas stutzeri*: From strain characteristics, extracellular polymers to transcriptomics

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Text S1. Preparation of PS NPs.

PS NPs were synthesized by emulsion polymerization [1, 2]. Specific operations were as follows: 0.08 g of $C_{12}H_{25}NaSO_4$, 0.11 g of $NaHCO_3$, 125 mL of deionized water, and 6 mL of purified styrene were added into a 250 mL three-necked flask. Then the mixture was thoroughly blended and assembled onto the reaction apparatus. N_2 was continuously energized, the stirring speed was controlled at 300 r/min, and the water bath heating was started. Start timing when the water bath temperature reached 75°C and at the 30th minute, 20 mL of 5.5 g/L aqueous ammonium persulfate solution was added, and the reaction was continuous for 5 h. The flask was rapidly cooled after the reaction. The cooled emulsion was transferred to a dialysis bag for dialysis for more than 48 h to remove the residual reactants. The dialyzed nanoplastic emulsion was stored at 2-8°C.

Text S2. Strain rejuvenation

Pseudomonas stutzeri (*P. stutzeri*) was inoculated into tryptone soy agar (TSA) medium and placed in a constant temperature oscillation incubator at 150 rpm, 28℃ for 24 h. Then the bacterial solution was mixed with an equal volume of 50% (v/v) sterile glycerol, and partitioned into 1.5 mL sterile freezing tubes to make glycerol bacterium, and the preserved glycerol bacterium was inoculated into TSA medium at 1% (v/v), placed in a constant temperature shaking incubator at 150 rpm and 28℃ for 24 h to facilitate revitalization. and then centrifuged at 4000 rpm and 4℃ for 10 min to collect the precipitate of *P. stutzeri*. *P. stutzeri* was resuspended with 0.01 M neutral phosphate buffered solution (PBS) and the cell density OD_{600} of the suspension was adjusted to approximately 1.0. The resulting suspension was used for subsequent inoculation.

Text S3. Compositions of mediums.

Tryptone Soy Agar (TSA) Medium： The TSA medium per litre used for the experiment was formulated as 15.0 g tryptone, 5.0 g soy peptone and 5.0 g NaCl, and the pH was adjusted to 7.0-7.5 with 1 mol/L HCl or NaOH solution. Then the medium was fixed in purified water and dispensed into conical flasks, autoclaved at 121℃ for 30 min. The solid medium was prepared by adding 15.0 g/L agar powder to this formula, and then dispensed into petri dishes after autoclaving.

Denitrification (DN) medium: the DN medium per liter used for the experiment was formulated as 0.72 g KNO_3, 3.0 g $C_6H_{12}O_6 \cdot H_2O$, 4.78 g $Na_2HPO_4 \cdot 12H_2O$, 1.24 g $NaH_2PO_4 \cdot 2H_2O$, 4.0 g NaCl and 3 mL of trace element solution. Where the trace elements per liter were formulated as 3.01 g $MgSO_4 \cdot 7H_2O$, 3.36 g $MnSO_4 \cdot H_2O$, 1.12 g H_3BO_3, 3.00 g $ZnSO_4 \cdot 7H_2O$, 0.30 g $FeSO_4 \cdot 7H_2O$, 0.6 g $CaCl_2$.

Table. S1 Characteristics of PS NPs and PS-NH_2 NPs.

	Partical size (nm)	Zeta potential (mV)	Solid content of emulsion (mg/mL)
PS NPs	85.2 ± 2.3	-24.8 ± 3.2	27.5
PS-NH_2 NPs	93.2 ± 1.5	19.0 ± 0.8	25.0

Table. S2 C, O, N and functional group composition in the EPS of *P. stutzeri.*

Groups	Elemental composition (molar ratio to C)		C 1s peak functional group relative content (%)		
	O/C	N/C	C-(C, H)	C-(O, N)	C=O/O-C-O
			284.08 eV	285.80 eV	287.67 eV
1	0.11	0.063	80.16	17.91	1.94
2	0.38	0.021	77.65	18.86	3.49
3	0.38	0.049	74.67	22.86	2.47
4	0.13	0.061	63.92	21.34	14.74
5	0.14	0.029	68.08	16.22	15.70

Note: Subgroups 1-5 represent the control group, PS 10 mg/L，PS 50 mg/L，PS-NH_2 10 mg/L，PS-NH_2 50 mg/L, respectively.

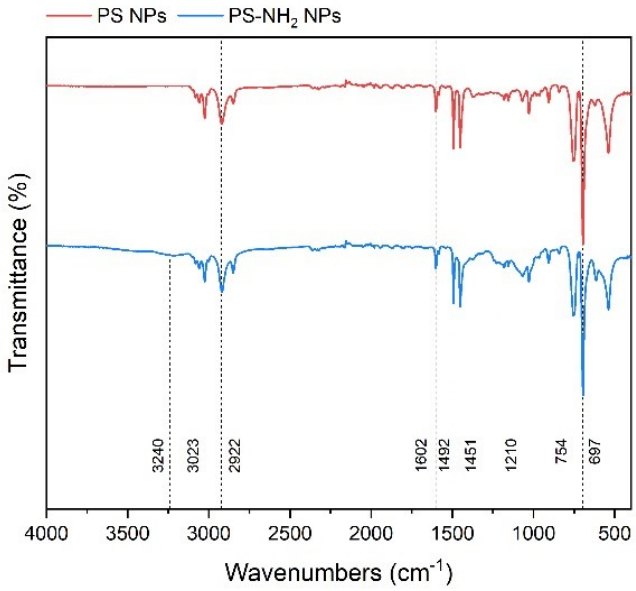

Fig. S1 FTIR spectra of PS NPs and PS-NH_2 NPs.

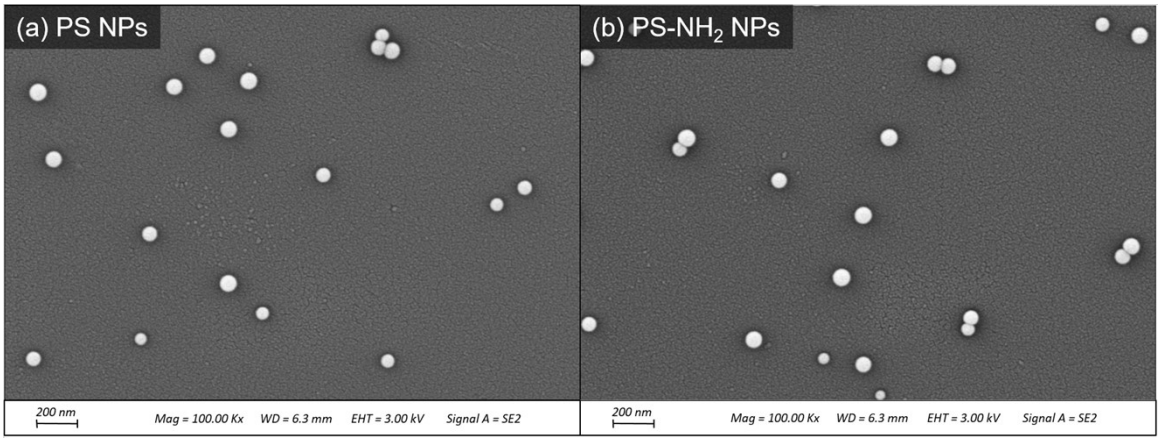

Fig. S2 SEM image of PS NPs (a) and PS-NH_2 NPs (b).

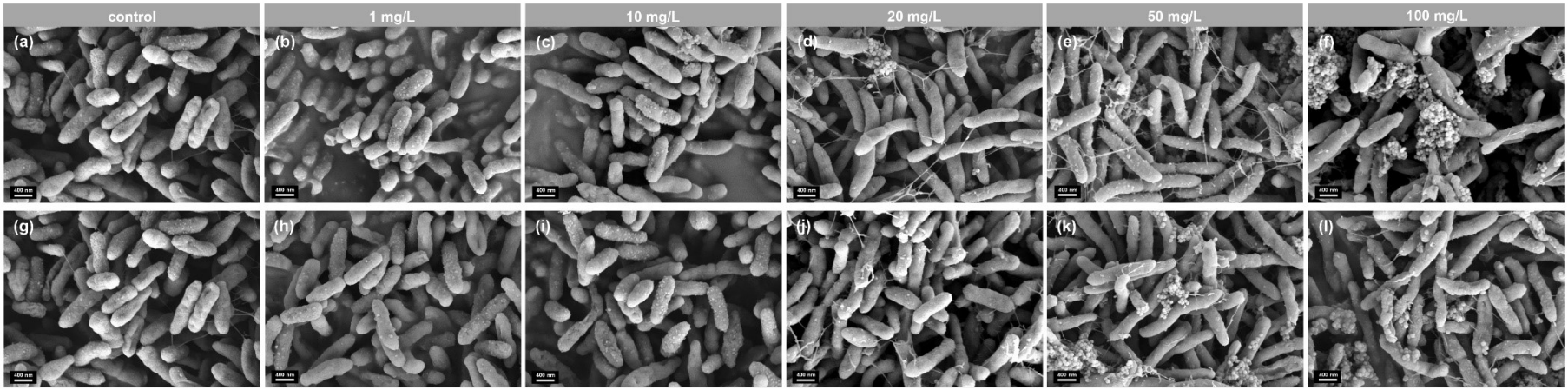

Fig. S3. SEM images of *P. stutzeri* under exposure to different concentrations (0, 1, 10, 20, 50, and 100 mg/L) of PS NPs (a–f) and PS-NH_2 NPs (g–l).

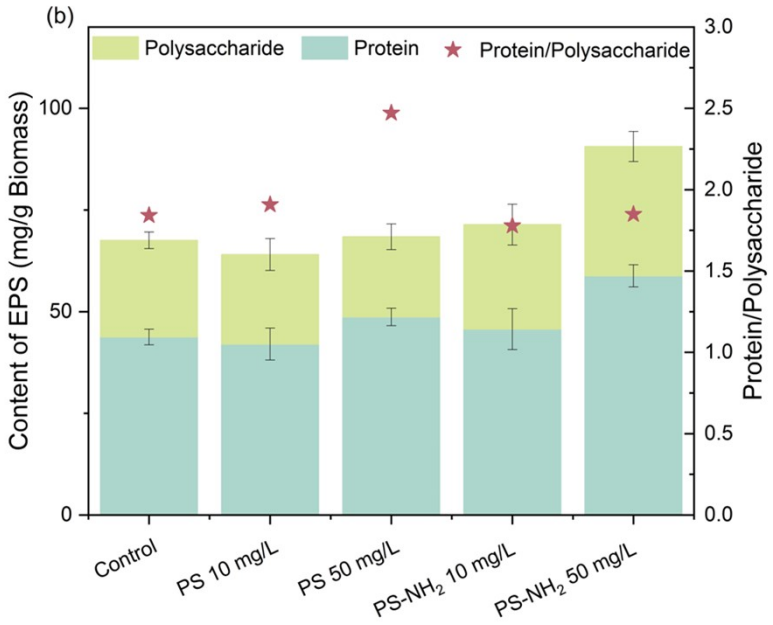

Fig. S4 EPS content and composition of *P. stutzeri* under the exposure to PS NPs and $PS\text{-}NH_2$ NPs.

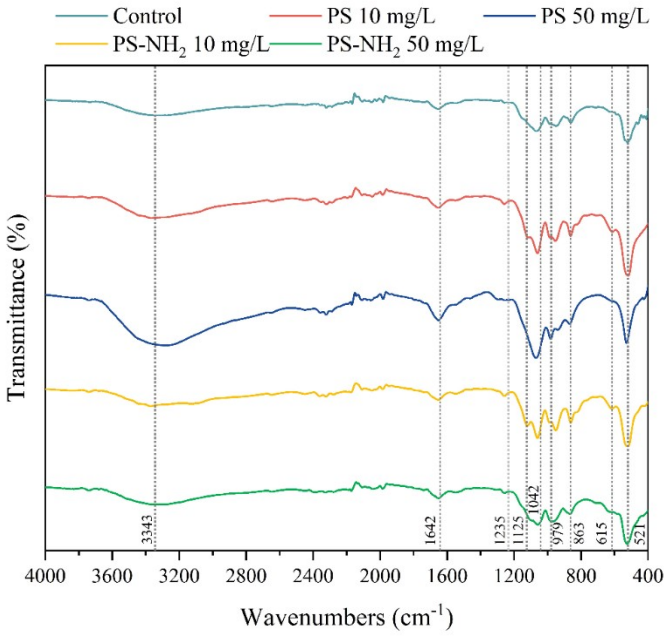

Fig. S5 Infrared spectra of EPS in *P. stutzeri* under the exposure to PS NPs and PS-NH_2 NPs.

References

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