Functionalized bacteria used as adsorbents for uranium extraction from seawater

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Supporting Information

1.Plasmid transformation and expression verification

The constructed Lpp–OmpA–Linker–U09–pBAD plasmid (Fig. S1) was introduced into *Escherichia coli* ECN 1917 competent cells via heat-shock transformation. Briefly, plasmid DNA was mixed with ECN 1917 competent cells and incubated on ice for 30 min, followed by heat shock at 42 °C for 45 s and immediate cooling on ice for 5 min. Fresh LB medium was then added, and the cells were allowed to recover at 37 °C with shaking at 200 rpm for 1 h. The recovered culture was spread onto LB agar plates containing 100 μg/mL ampicillin and incubated at 37 °C for approximately 16 h to obtain positive transformants.

To verify recombinant protein expression on the surface of ECN 1917, the transformed strain was inoculated into LB medium supplemented with 100 μg/mL ampicillin and cultured at 37 °C until reaching the logarithmic growth phase. L-arabinose was then added to a final concentration of 0.2% to induce protein expression, and the culture was further incubated at 25 °C overnight.

Cells were harvested by centrifugation (4 °C, 6000 rpm, 5 min), and total protein was extracted. Protein concentration was determined using a bicinchoninic acid (BCA) assay. Extracted proteins

were separated by SDS–PAGE and transferred onto a PVDF membrane. After blocking with 0.5% skim milk for 1 h, the membrane was incubated with an HRP-conjugated anti-His monoclonal antibody (1:5000, Proteintech) for 1 h. Protein bands were visualized using an enhanced chemiluminescence reagent (ECL, Servicebio) to confirm target protein expression.

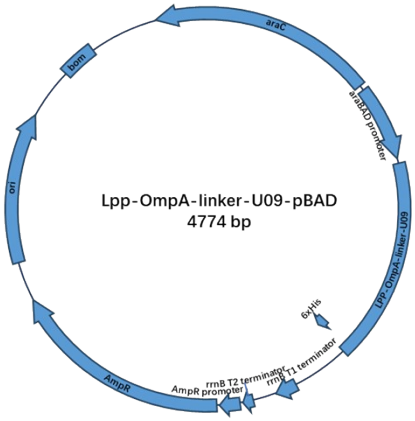

Fig. S1 Schematic illustration of the Lpp–OmpA–Linker–U09–pBAD plasmid.

2. Amino acid sequence of the Lpp–OmpA–linker–U09 fusion protein

Lpp-OmpA:
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAG
GTTGCTCCAGCAACGCTAAAATCGATCAGAACAACAATGGCCCGACCCATGAAAACC
AACTGGGCGCTGGTGCTTTTGGTGGTTACCAGGTTAACCCGTATGTTGGCTTTGAAAT
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GCGATCACTCCTGAAATCGCTACCCGTCTGGAATACCAGTGGACCAACAACATCGGT
GACGCACACACCATCGGCACTCGTCCGGACAAC

U09:
CTGGATTGCCGTGAACGCATTGAAAAAGACCTGGAAAACCTGGAAAAAGAACTGATG
GAAATGAAAAGCATCAAACTGTCTGATGACGAAGAAGCGGTGGTTGAACGTGCCCTG
AATTATCGCGATGACAGTGTCTATTACCTGGAAAAAGGCGATCATATTACCTCCTTTG
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Linker:
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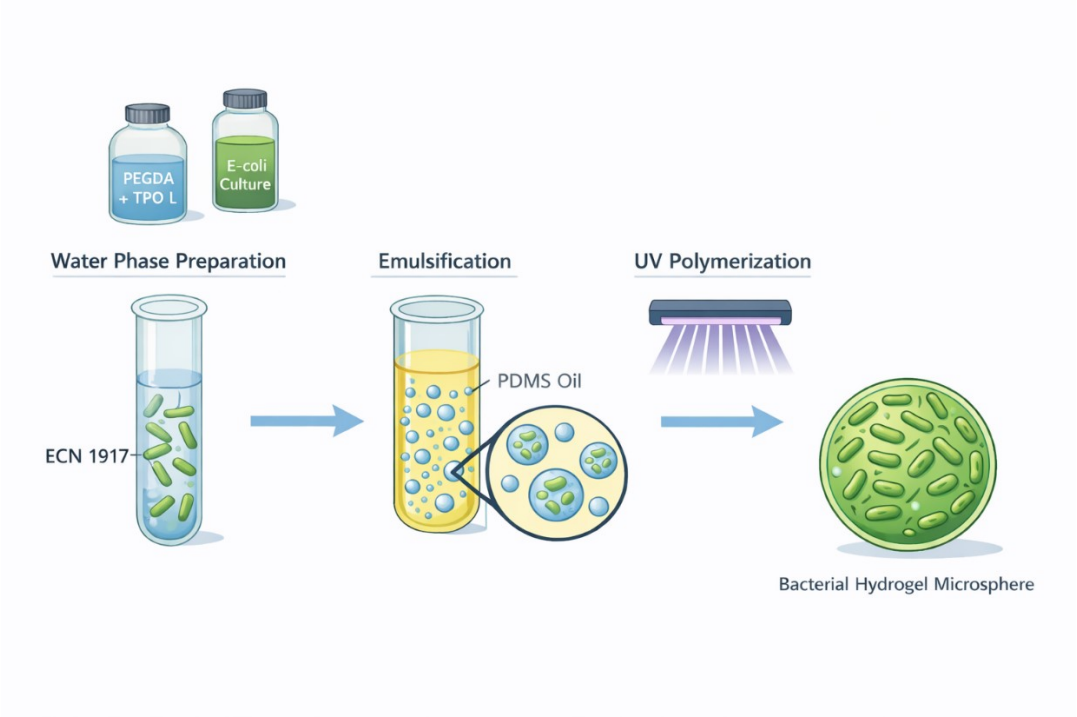


Fig. S2 Schematic illustration of microbial beads fabrication using ECN 1917.

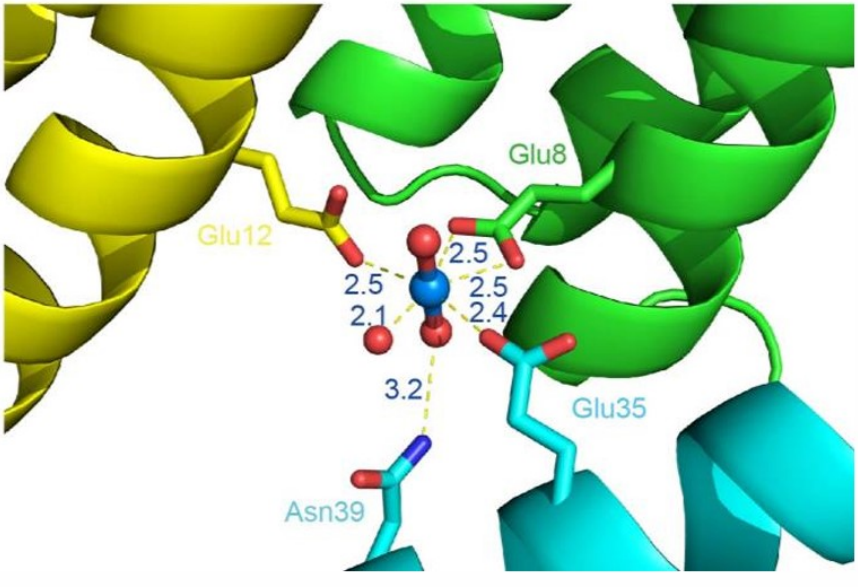


Fig. S3 Schematic illustration of the binding interaction between U09 and uranyl ions.[1]

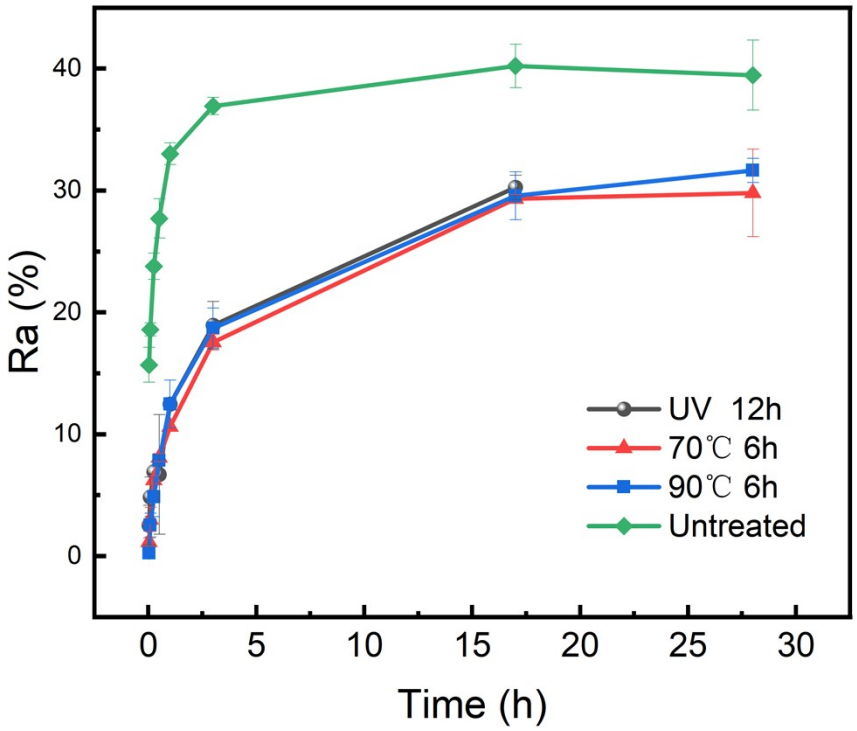


Fig. S4 The influence of different treatment methods on the adsorption of U(VI) by microbial beads

Table S1 The separation factors ($SF_{U/M}$) of U and other metals in spiked simulated seawater

adsorbent	Ca	K	Mg	Na	U	Cd	Zn	Eu
BDU09	7.4	10.2	6.8	7.9×10^3	1	19.0	7.9×10^3	2.7
microbial beads	5.9×10^3	67.3	5.9×10^3	3.7×10^3	1	74.5	5.9×10^3	3.3×10^3

Table S2 The component of simulated seawater and natural seawater

	K	Sr	U	Ca	Mg	Na
Spiked natural seawater	159.35	14.28	11.95	319.30	970.50	8605
Spiked simulated seawater	892.00	8.03	14.85	279.18	774.50	6440

Spiked natural seawater: natural seawater was collected from the Bohai Sea. A U(VI) stock solution was added to 500 mL of seawater previously filtered through a 0.22 μm membrane to obtain a final U(VI) concentration of 5.00×10^{-5} mol/L. The pH was adjusted to 8.2. Subsequent procedures were identical to those used for the spiked simulated seawater experiments.

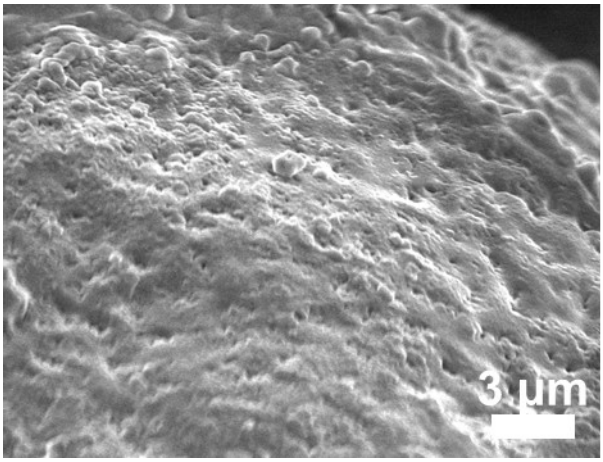


Fig. S5 SEM images of microbial beads after five adsorption–desorption cycles.

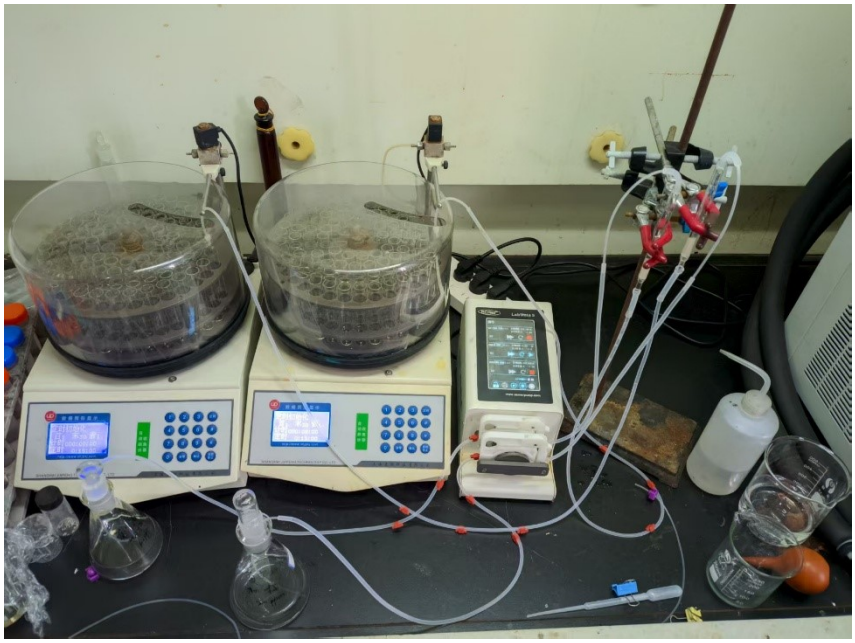
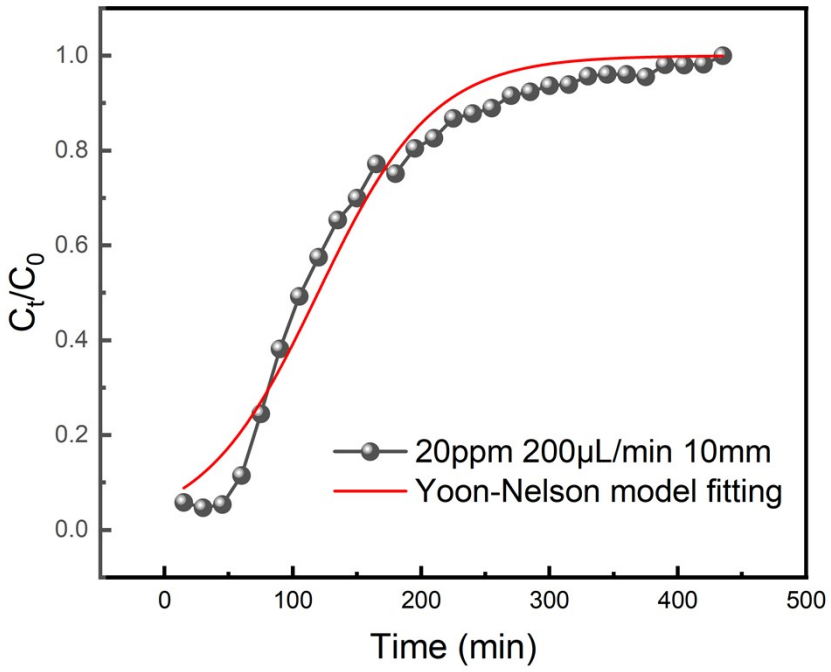
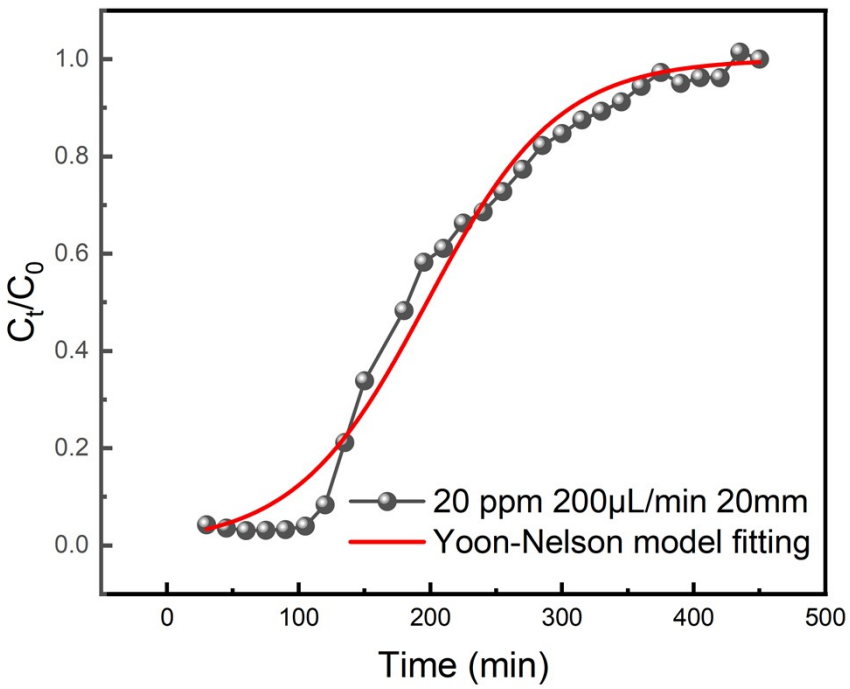


Fig. S6 Column experiment equipment of U(VI) separation by microbial beads

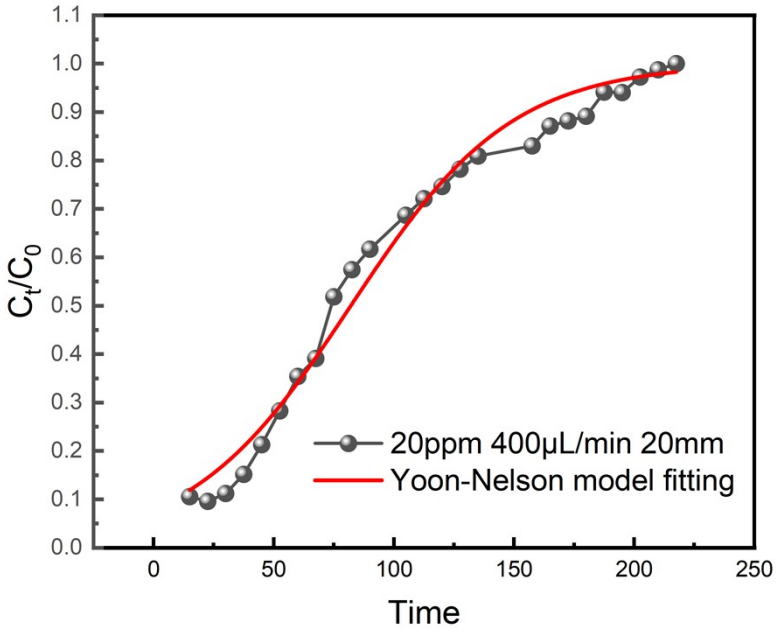
a)


1.0
0.8
0.6
0.4
0.2
0.0
C_t/C_0
0
100
200
300
400
500
Time (min)
20ppm 200μL/min 10mm
Yoon-Nelson model fitting

b)


1.0
0.8
0.6
0.4
0.2
0.0
C_t/C_0
0
100
200
300
400
500
Time (min)
20 ppm 200μL/min 20mm
Yoon-Nelson model fitting

c)



d)

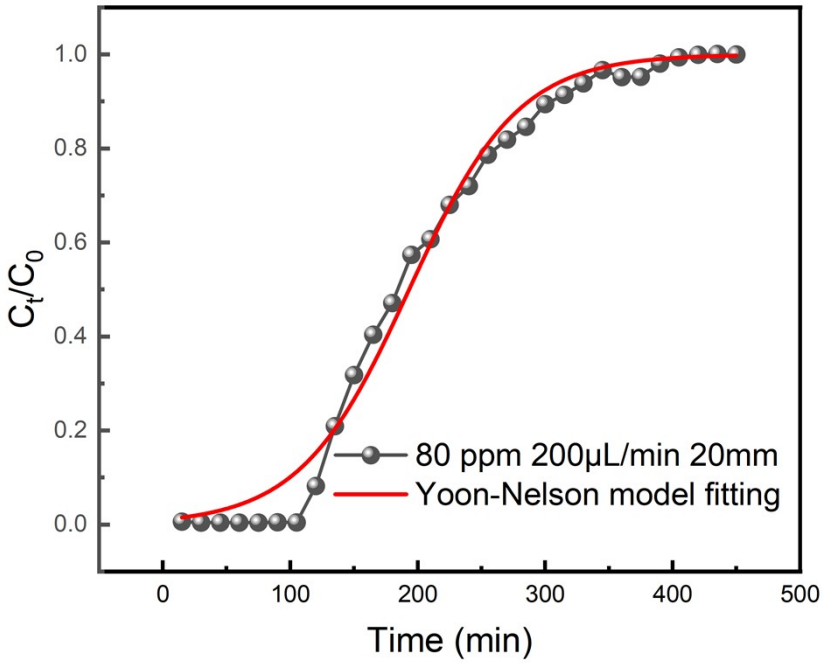


Fig. S7 Experimental and Theoretical breakthrough curve predicted by YN model. (The experimental conditions shown in the figure are U(VI) concentration (ppm), flow rate (μL min^{-1}), and column height (mm)).

Reference

[1] Zhou, L., et al. (2014). "A protein engineered to bind uranyl selectively and with femtomolar affinity." Nature Chemistry **6**(3): 236-241.doi.org/10.1038/nchem.1856