Supporting Information

Superior performance of natural electron shuttles over a synthetic analogue in promoting electrogenic hydrocarbon degradation in contaminated soil

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Table S1. Experimental design summary.

Treatment Group	Electron Shuttle Addition	Overlying Water	Circuit	Label	Replicates	Sampling Time
Control	/	Distilled water	No electrode	CK-NE	4	10 d
			Open circuit	CK-OC		88 d
			Closed circuit	CK-CC		
With Electron Shuttle Addition	10 ppm Anthraquinone-2,6-disulfonate (AQDS)	Distilled water	No electrode	AQDS-NE	4	10 d
			Open circuit	AQDS-OC		88 d
			Closed circuit	AQDS-CC		
	10ppm Phenazine (PHE)	Distilled water	No electrode	PHE-NE	4	10 d
			Open circuit	PHE-OC		88 d
			Closed circuit	PHE-CC		
	10 ppm L-Cysteine (CYS)	Distilled water	No electrode	CYS-NE	4	10 d
			Open circuit	CYS-OC		88 d
			Closed circuit	CYS-CC		
Control + Glucose	/	0.5% Glucose solution	No electrode	CK-GLU-NE	2	88 d
			Open circuit	CK-GLU-OC		
			Closed circuit	CK-GLU-CC		
With Electron Shuttle Addition+ Glucose	10 ppm Anthraquinone-2,6-disulfonate (AQDS)	0.5% Glucose solution	No electrode	AQDS-GLU-NE	2	88 d
			Open circuit	AQDS-GLU-OC		
			Closed circuit	AQDS-GLU-CC		
	10 ppm Phenazine (PHE)	0.5% Glucose solution	No electrode	PHE-GLU-NE	2	88 d
			Open circuit	PHE-GLU-OC		
			Closed circuit	PHE-GLU-CC		
	10ppm L-Cysteine (CYS)	0.5% Glucose solution	No electrode	CYS-GLU-NE	2	88 d
			Open circuit	CYS-GLU-OC		
			Closed circuit	CYS-GLU-CC		

Text S1. The determination method of EDC and EAC.

The determination was performed using an electrochemical workstation coupled with a three-electrode system, comprising an Ag/AgCl reference electrode, a glassy carbon working electrode, and a platinum wire counter electrode(Feng et al., 2025). For the EDC test, the set redox potential is 0.61 V. In an electrolytic cell containing 2.5 mL of phosphate buffer solution (pH=7) and 25 mL of potassium chloride solution (0.2 mol/L), the prepared electrode is inserted and connected to the workstation. The "Amperometric *i-t* curve parameters" mode (4000 s) is selected to start the baseline detection (600 s). After stabilization, 1 mL of the mediator 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (4 g/L) is added. When the reaction stabilizes (600 s), the target solution is introduced. For the EAC test, the redox potential is -0.49 V, and the mediator is diquat dibromide hydrate (2 g/L). The calculation formula is EDC or EAC = Ap / (e × MC × NA), where Ap represents the integrated charge (from the oxidation or reduction curve), e is 1.6×10^{-19} C, MC is the carbon content in the system, and NA is Avogadro's constant (6.02×10^{23} mol^{-1}).

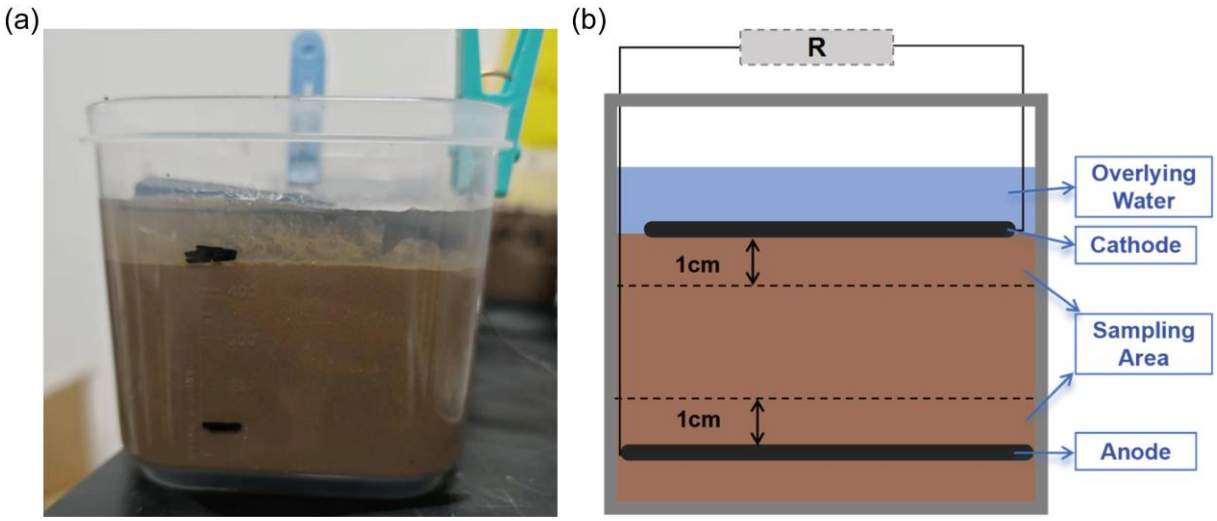

Figure S1. Physical diagram of the MES reactor (a) and schematic diagram of MES reactor sampling (b).

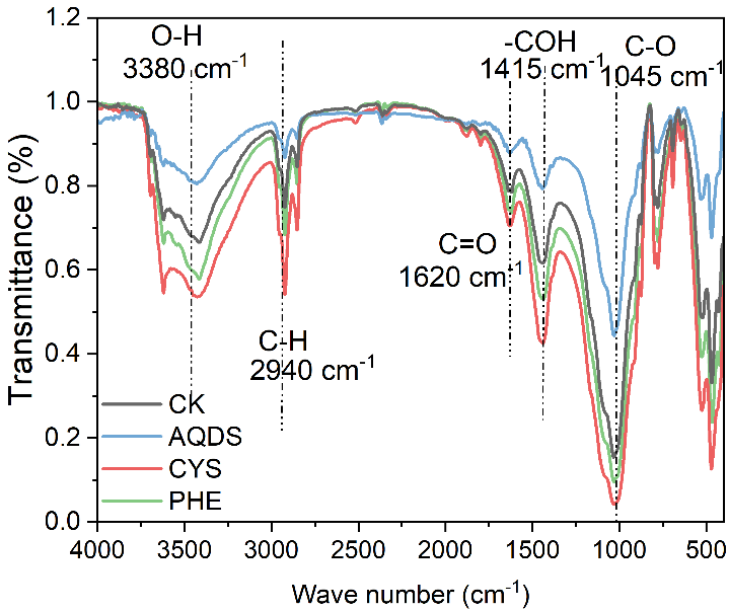

Figure S2. FTIR spectra of soil samples after incubation with different electron shuttles.

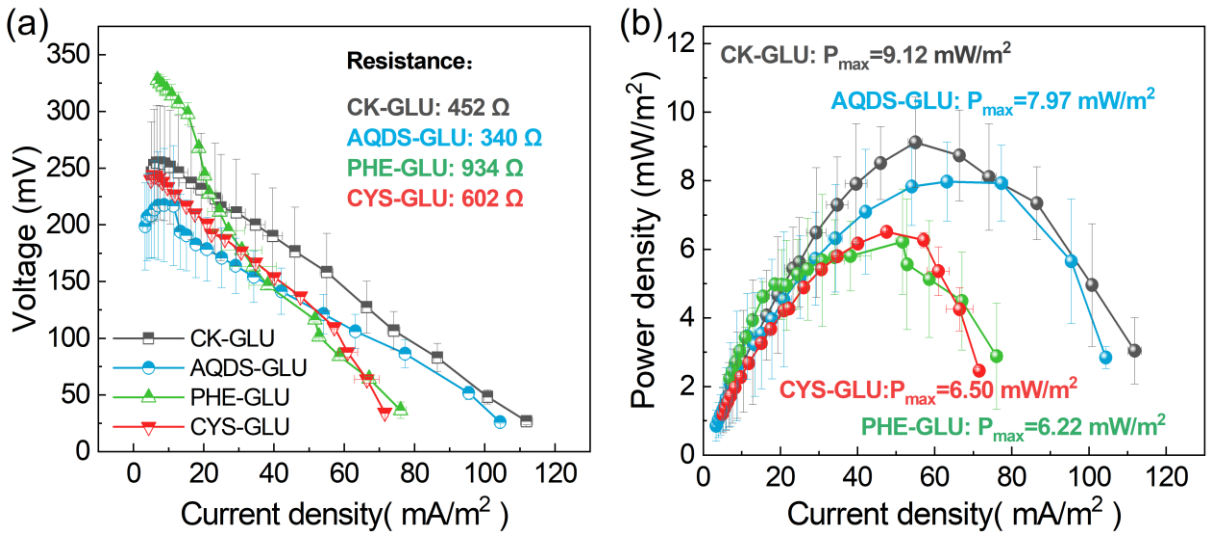

Figure S3. Electrochemical performance of glucose-supplemented groups. (a) Polarization curves, (b) Power density curves.

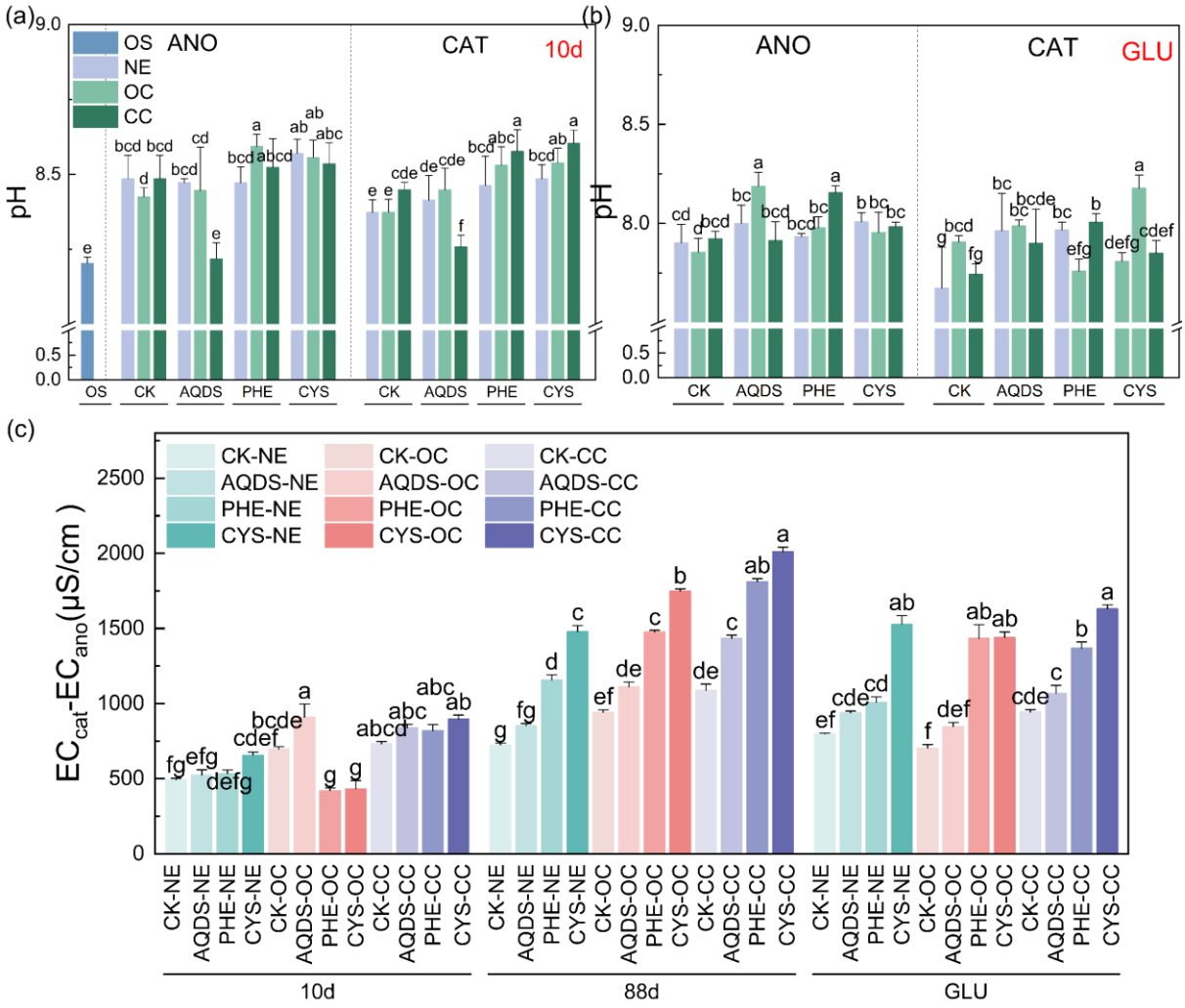

Figure S4. Electrical conductivity and pH between the anode and cathode. (a) pH values measured on day 10, (b) pH values of glucose-supplemented groups, (c) Difference in conductivity between the anode and cathode.

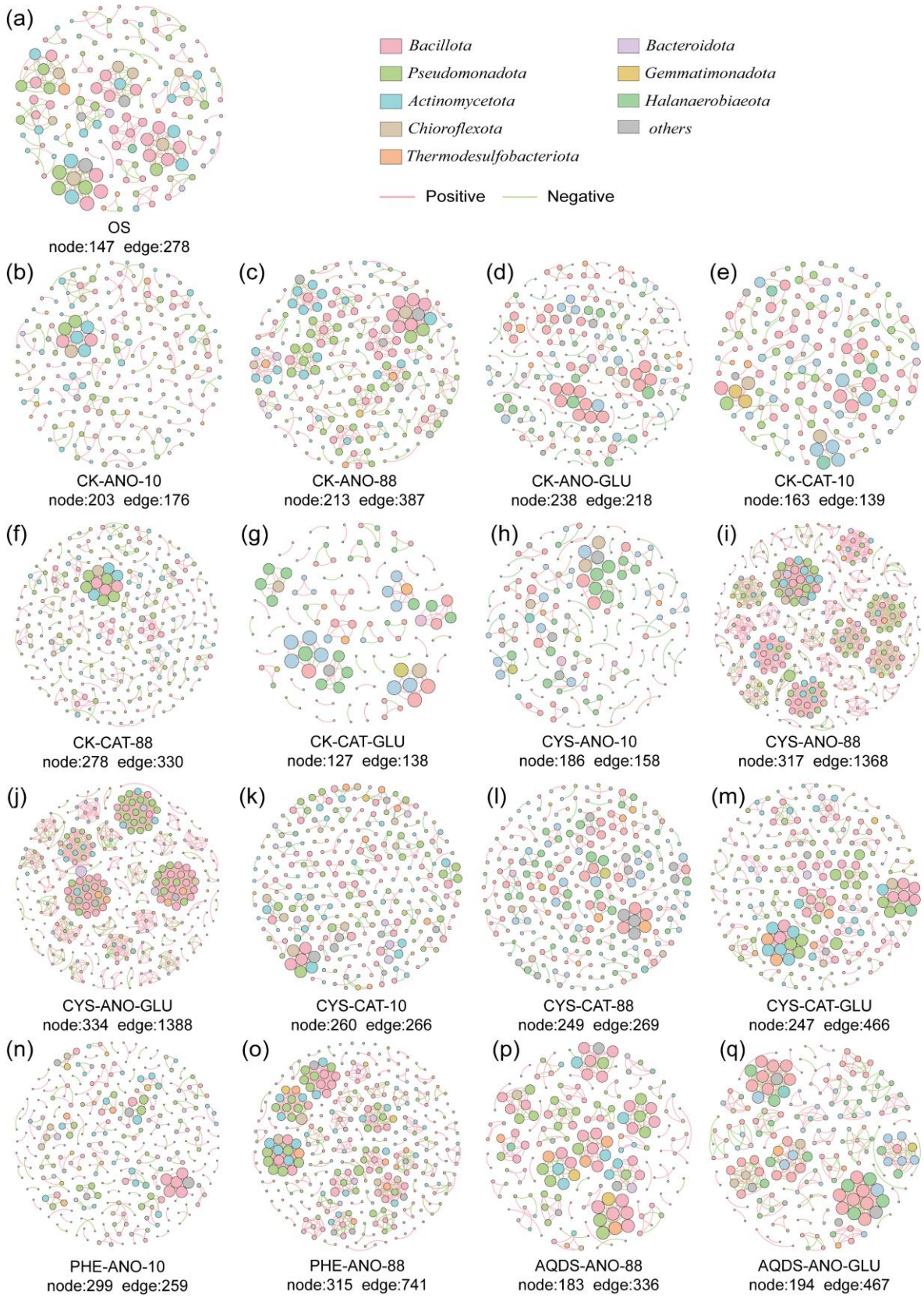

Figure S5. Bacterial symbiotic networks across all groups ($p < 0.05$). Circles represent bacterial taxa, with node size proportional to connection number. Pink and green denote positive and negative correlations, respectively.

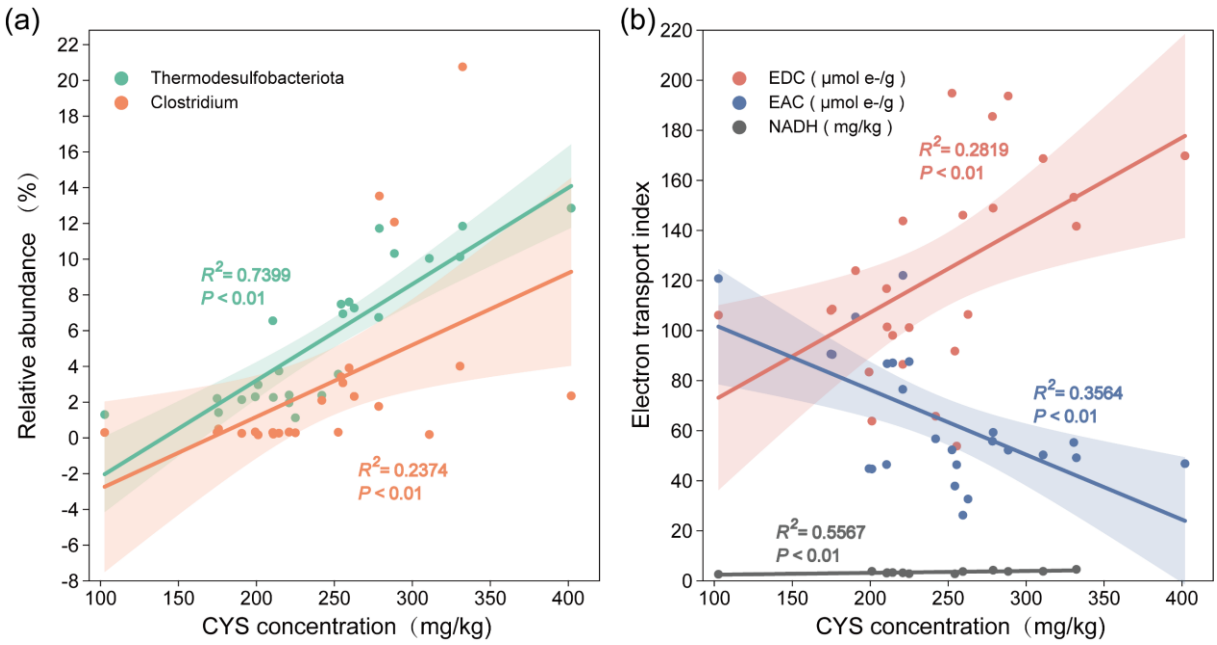

Figure S6 Linear regression analysis of cysteine concentration versus the relative abundance of core functional microbiota (a), and linear regression analysis of cysteine concentration versus key electron transfer indicators (EDC, EAC, NADH) (b).

Reference

Feng, X., Li, Y., Jin, J., Qiao, W., Gao, Z., Guo, H. 2025. Electrochemistry and Molecular Compositions Reflect Electron Shuttling of Dissolved Organic Matter in High Arsenic Groundwater. *Environmental Science & Technology*, **59**(17), 8591-8601.