# ELECTRONIC SUPPLEMENTARY INFORMATION

## Biological Anolyte Regeneration System for Redox Flow Batteries

Olja Simoska,[1,a] Zayn Rhodes,[1] Emily Carroll,[1] Katia N. Petrosky,[1] Shelley D. Minteer[1]*

[1]*Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States*

[a] *Current address: Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, United States*

*Corresponding author: Shelley D. Minteer (minteer@chem.utah.edu)

**Experimental methods.**

***Chemicals.*** All reagents and chemicals were used as received. Pyocyanin and phenazine-1-carboxylic acid were purchased from Cayman Chemical Company. Ethanol, 3-(N-morpholino)propane sulfonic acid (MOPS), and kanamycin were acquired from Sigma-Aldrich. D-glucose (glucose), lysogeny broth, and magnesium chloride ($MgCl_2$) were obtained from Fisher Scientific Inc. Further, (ferrocenylmethyl)trimethylammonium was synthesized using a literature procedure and characterization was in good agreement with published results.[1]

***Cell culture and microbial growth.*** The *Escherichia coli phzAG-SM* strain was genetically engineered in our research laboratory. *E. coli* bacterial cells were cultured from freezer stock on LB agar plates with kanamycin resistance. Using *E. coli* initially grown on LB agar plates, overnight liquid cultures were prepared by inoculating 250 mL cell culture flasks filled to a volume of 150 mL with liquid LB growth medium with kanamycin resistance. Bacterial cells were grown in a 37 °C incubator for 16h with a measured optical density at 600 nm (OD600) of 1. After this 16-h bacterial culture growth, the *E. coli phzAG-SM* cells were collected by centrifugation at 5000× g (Allegra X-15R Benchtop Centrifuge, Beckman Coulter) for 20 min. In the following washing step, the bacterial cells were resuspended in 1 mL of 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 200 mM glucose and further concentrated by centrifugation at 15,000 rpm (Eppendorf Centrifuge 5424 R) for 10 min. In the final step, a solution with a bacterial cell concentration of 1 g $mL^{-1}$ of *E. coli phzAG-SM* was prepared using 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 200 mM glucose. For cyclic voltammetric experiments and redox flow battery cycling tests, *E. coli phzAG-SM* cells were immobilized on AvCarb carbon paper (AvCarb MGL 190, Fuel Cell Store). The cut carbon papers were sterilized via exposure to UV light. Using the prepared 1 g $mL^{-1}$ bacterial solution, 150 µL bacterial cells were deposited on carbon paper surfaces. The solution on the electrodes was allowed to dry under an $N_2$ gas atmosphere for 1 h. The dry AvCarb carbon paper with immobilized *E. coli phzAG-SM* cells were either used as the working electrode in time-dependent cyclic voltammetry experiments or introduced in the anolyte container for RFB experiments. It is important to note that the carbon paper was not used as the working electrode in RFB cycling experiments; however, it was used as immobilization support for the *E. coli phzAG-SM* cells.

***Electrochemical setup for time-dependent cyclic voltammetry tests.*** A 2 mM stock solution of phenazine-1-carboxylic acid (PCA) was 20 mM MOPS buffer (pH 7) containing 10 mM $MgCl_2$ and 200 mM glucose as the aqueous solutions. The 2 mM PCA stock solution was initially stabilized with 20% ethanol, giving a final concentration below 0.1% in the electrolyte solution. Specifically, the 2 mM stock PCA solution was diluted to 500 µM PCA solution in the aqueous MOPS buffer solution. Time-dependent cyclic voltammetric (CV) tests were performed using a three-electrode cell setup at room temperature (20 ± 1°C). The electrochemical CV responses were examined using a CH660 potentiostat (CH Instruments). For the time-dependent CV analysis, the working electrodes utilized were AvCarb carbon paper electrodes (AvCarb MGL 190, Fuel Cell Store) with an area of 1 $cm^2$ with or without immobilized *E. coli phzAG-SM* cells, and the counter electrode was a Pt mesh electrode. The electrolyte solution used for electrochemical experiments was 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 200 mM glucose. CV tests were performed at a scan rate of 10 mV $s^{-1}$.

***RFB cell construction and testing.*** All RFB tests were performed in an oxygen-free glovebox using a BioLogic VSP (BioLogic Sciences Instruments) and utilizing a zero-gap flow cell[2] with an anion exchange membrane (Selemion AMVN) and graphite flow field plates with carbon paper to increase the RFB electrode surface area. RFB cells were cycled at 0.2 mA charge and –0.1 mA discharge currents, with a potential limit of 0–1.2 V vs. $Ag/Ag^+$ for 10 cycles for the phenazine-1-carboxylic acid/(ferrocenylmethyl)trimethylammonium (PCA/FcN) (anolyte/catholyte) phenazine RFB cells. The anolyte tank included 500 μM PCA in solution two sets of 150 mg of immobilized *E. coli phzAG-SM* cells on carbon paper support, and the catholyte included a 1000 μM FcN solution. Control experiments were performed with (1) 500 μM PCA without *E. coli phzAG-SM* cells, and (2) *E. coli phzAG-SM* cells without 500 μM PCA, as the anolyte. Tests were conducted in the 20 mM MOPS (pH 7) + 10 mM $MgCl_2$ + 200 mM glucose buffer with 0.2 mA charge and -0.1 mA discharge current in order to access a higher charge state and give the genetically engineered cells sufficient time to begin the synthesis of phenazine species. For the pre- and post-cycling cyclic voltammograms, a glassy carbon electrode with a 3 mm diameter was used as the working electrode, a Pt wire was used as the counter electrode, and a quasi-$Ag/Ag^+$ silver wire was utilized as the reference electrode. Pre- and post-cycling cyclic voltammograms were obtained at scan rates of 100 mV $s^{-1}$. For RFB testing attempted at higher charging and discharging rates, RFB cells were cycled at 1 mA charge and –0.5 mA discharge currents, with a potential limit of 0–1.2 V vs. $Ag/Ag^+$ for 10 cycles for PCA/FcN (anolyte/catholyte) phenazine RFB cells.

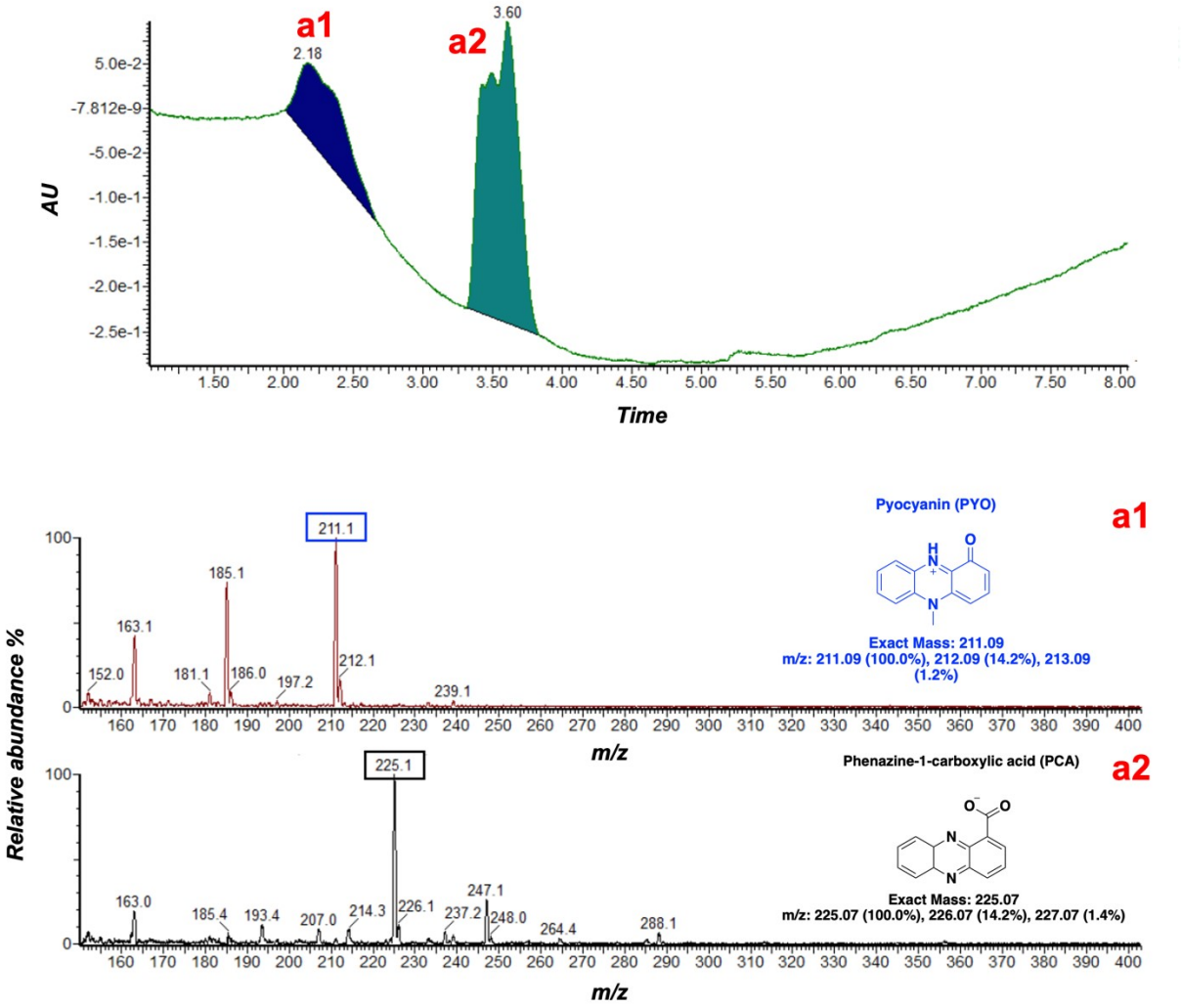



**Fig. S1** Liquid chromatography-mass spectrometry (LC-MS) data obtained the supernatant of the genetically engineered *E. coli phzAG-SM* cells showing two separation peaks with retention times of 2.18 (*a1*) and 3.60 min (*a2*), which correspond to PYO and PCA, respectively, as demonstrated by the m/z fragmentation pattern. This MS data confirms the identity of phenazine metabolites produced by the engineered *E. coli* cells.

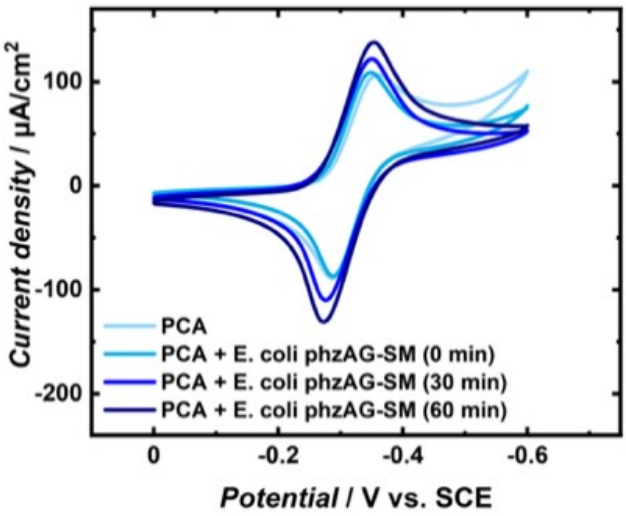
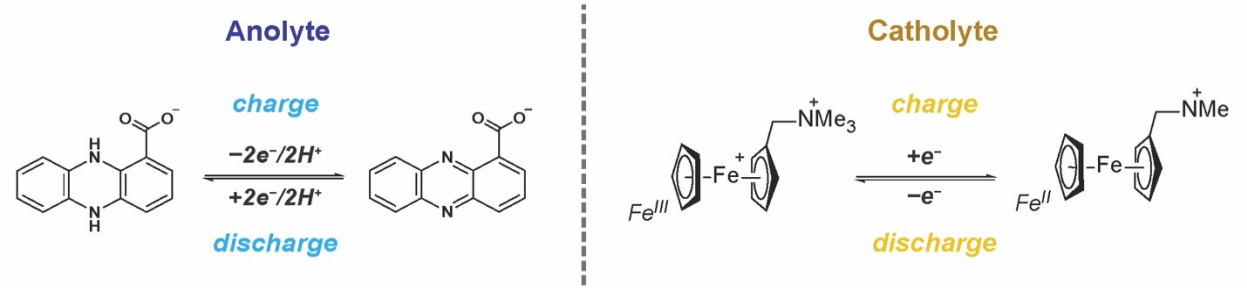



**Fig. S2** Time-dependent cyclic voltammetric responses of phenazine ROMs, namely PCA, generated from genetically modified *E. coli phzAG-SM* microbes immobilized on AvCarb carbon paper electrodes. Enhancement in the current density correlates to an increase in the phenazine concentrations in solution over time.




**Scheme S1.** Chemical structures and redox mechanisms of the PCA anolyte and FcN catholyte in the PCA/FcN RFB cell.

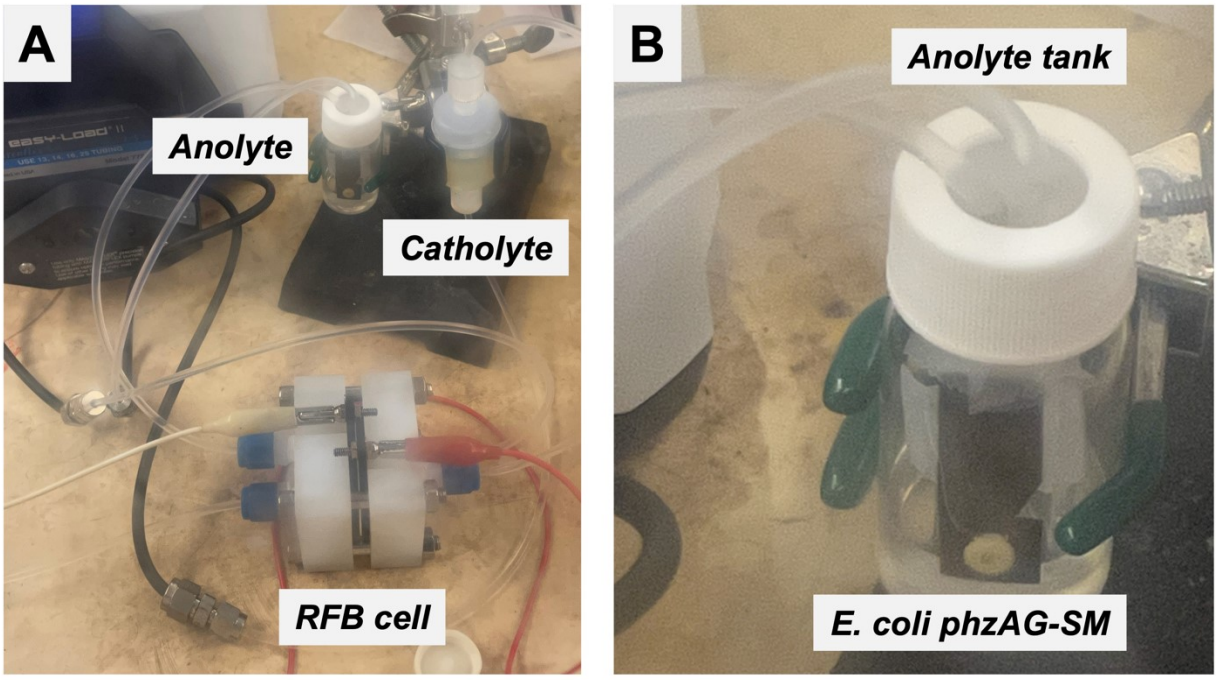



**Fig. S3** Redox flow battery (RFB) system setup for the showing (A) the PCA anolyte tank and the FcN catholyte tank. (B) Close-up image of the PCA anolyte tank containing the immobilized engineered *E. coli phzAG-SM* cells (yellow).

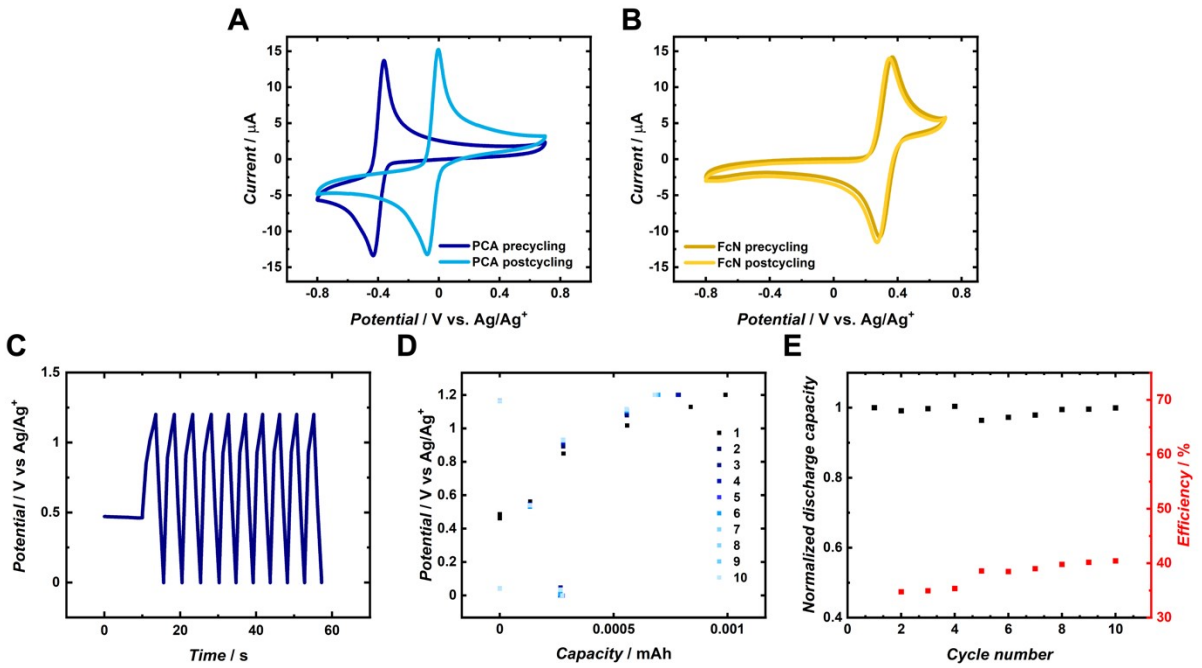



**Fig. S4** Results for a redox flow battery (RFB) system with *E. coli phzAG-SM* cells and with 500 µM phenazine-1-carboxylic acid (PCA) as the anolyte and 1000 µM (ferrocenylmethyl)trimethylammonium (FcN) as the catholyte, in 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 200 mM glucose at higher charging and discharging rates, specifically 1 mA charge and –0.5 mA discharge currents. Pre- and post-battery cycling cyclic voltammograms for (A) PCA anolyte and (B) FcN catholyte solutions. (C) RFB cycling data and (D) charge/discharge curves, for 10 cycles. (E) Normalized discharge capacity for the PCA/FcN RFB cell and cell cycling Coulombic efficiency.

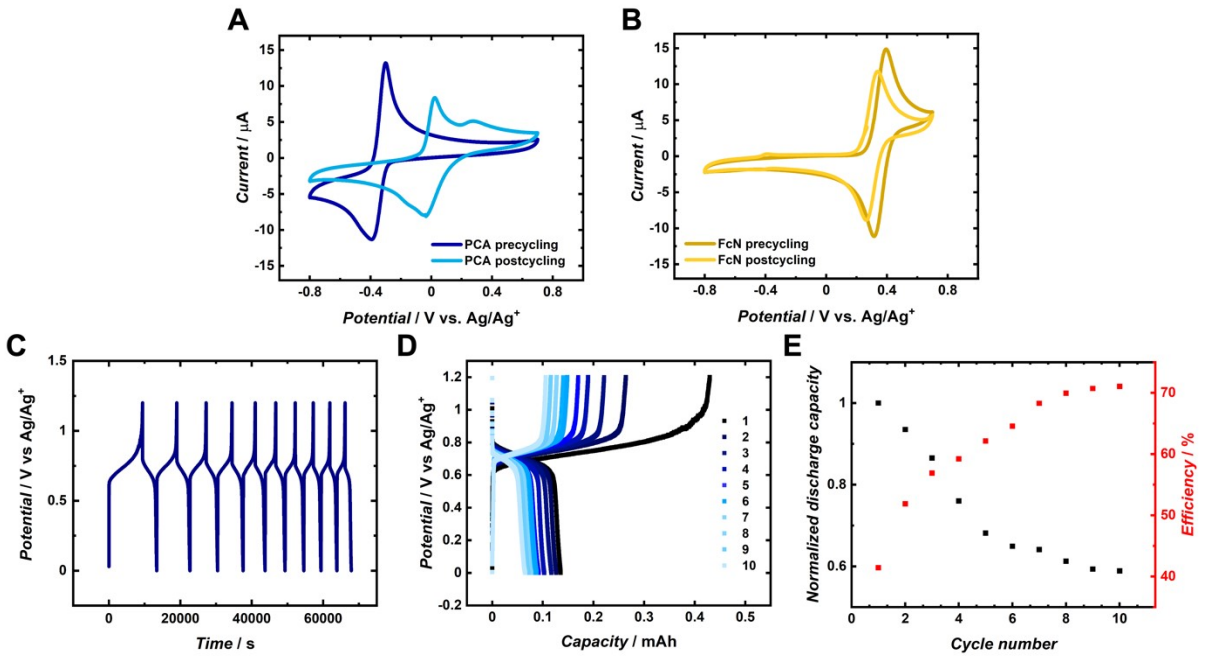



**Fig. S5** Results for a redox flow battery (RFB) system with 500 µM phenazine-1-carboxylic acid (PCA) without *E. coli phzAG-SM* cells as the anolyte and 1000 µM (ferrocenylmethyl)trimethylammonium (FcN) as the catholyte, in 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 200 mM glucose. Pre- and post-battery cycling cyclic voltammograms for (A) PCA anolyte and (B) FcN catholyte solutions. (C) RFB cycling data and (D) charge/discharge curves, for 10 cycles. (E) Normalized discharge capacity for the PCA/FcN RFB cell and cell cycling Coulombic efficiency. The structural identity of the additional redox peak at a more positive potential vs. $Ag/Ag^+$ in the PCA post-cycling data remains elusive as evidenced by the mass spectrometry data in Fig. S6.

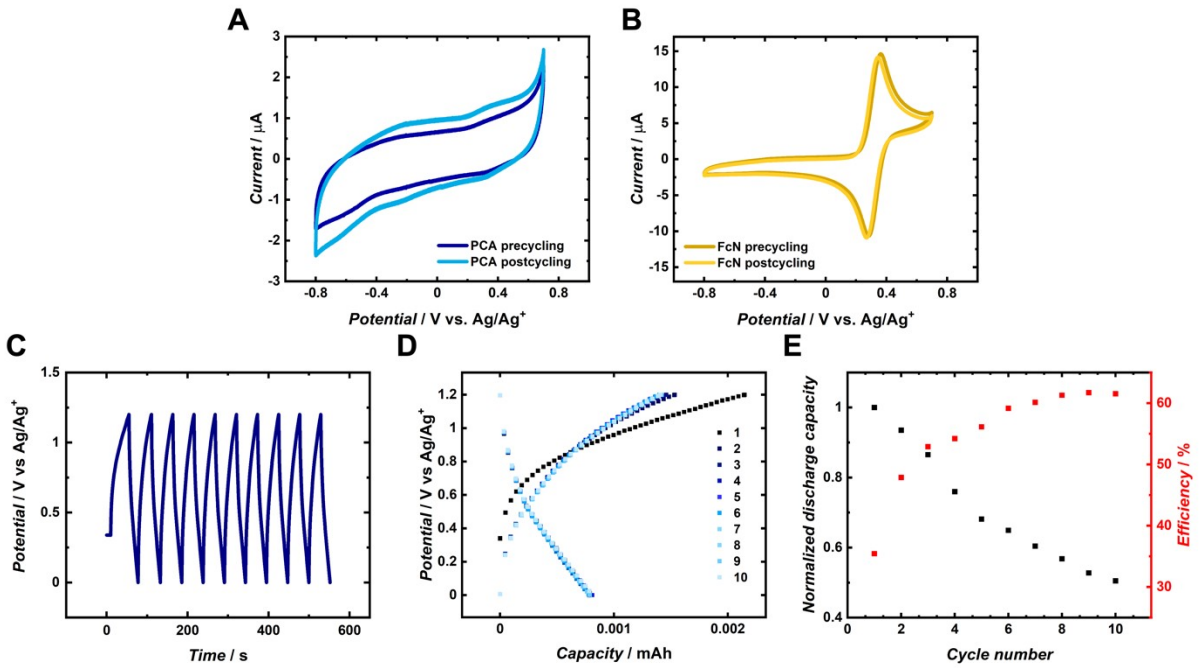



**Fig. S6** Results for a redox flow battery (RFB) system with *E. coli phzAG-SM* cells and without 500 µM phenazine-1-carboxylic acid (PCA) as the anolyte and 1000 µM (ferrocenylmethyl)trimethylammonium (FcN) as the catholyte, in 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 200 mM glucose. Pre- and post-battery cycling cyclic voltammograms for (A) PCA anolyte and (B) FcN catholyte solutions. (C) RFB cycling data and (D) charge/discharge curves, for 10 cycles. (E) Normalized discharge capacity for the PCA/FcN RFB cell and cell cycling Coulombic efficiency.

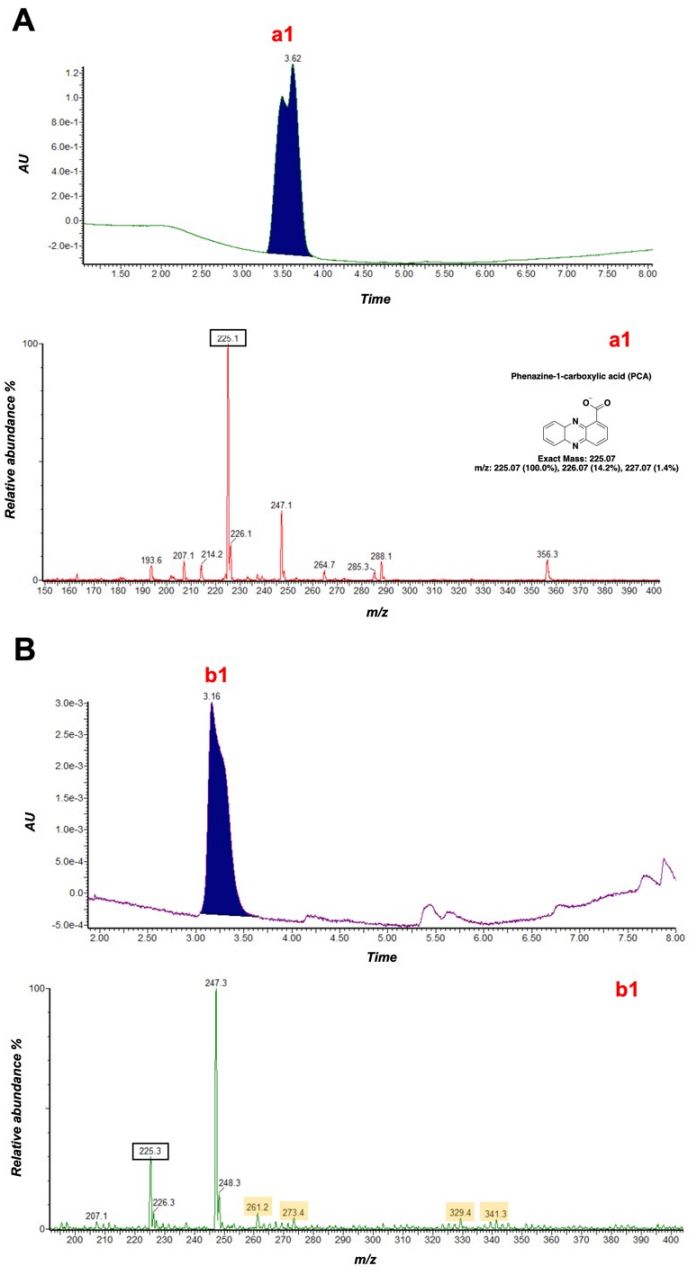



**Fig. S7** Liquid chromatography-mass spectrometry (LC-MS) data obtained from the RFB PCA anolytes (A) with and (B) without the genetically engineered *E. coli phzAG-SM* cells. The peaks with retention times of (A) 3.62 (*a1*) and (B) 3.16 min (*b1*) correspond to the PCA anolytes with and without *E. coli phzAG-SM* cells, respectively. The MS for the RFB post-cycling PCA anolyte containing *E. coli phzAG-SM* cells in (A) shows the presence of PCA by the 225 m/z fragmentation. The MS data for the RFB post-cycling PCA anolyte without *E. coli phzAG-SM* cells in (B) show the presence of PCA by the 225 m/z fragmentation, but at a lower relative abundance compared to (A) when the engineered *E. coli* cells are present. This qualitative data demonstrates the ability of the engineered *E. coli phzAG-SM* cells to regenerate PCA in the anolyte tank. Additional peaks present in the data in (B) are highlighted in yellow to show potential species that could correspond to degradation products present in the anolyte tank post-cycling when phenazine-producing cells are not present in the anolyte.

**References.**


1. B. Hu, C. DeBruler, Z. Rhodes and T. L. Liu, *J. Am. Chem. Soc.*, 2017, **139**, 1207-1214.
2. J. D. Milshtein, J. L. Barton, R. M. Darling and F. R. Brushett, *J. Power Sources*, 2016, **327**, 151-159.