Review of “Cr(VI) reduction, electricity production, and microbial resistance variation in paddy soil under microbial fuel cell operation” by Niu et al. for consideration in EGUsphere.

General comments

Niu et al. constructed a soil MFC to reduce Cr(VI) in paddy soil and investigate its impact on the microbial community and microbial resistance traits. The findings provide some important clues supporting the application of bioelectrochemical systems in contaminated paddy soils, offering a potential approach for environmental remediation and detoxification. However, several details require further improvement before being considered for publication.

We thank the reviewers for their time and feedback on this manuscript. Our point-to-point responses are below.

Specific comments

1. **Please explain why aluminum foam was chosen over other materials and what advantages it offers in this experiment.**

Response： Thank you for your suggestion.

In this experiment, the anode is operated in a low oxygen moist soil environment, and the soil medium composition is complex, requiring the anode to have high corrosion resistance. Porous metal skeletons, such as foam copper, foam nickel and foam aluminum, are known for their good conductivity and ability to evenly distribute the metal-ion flux through spatial segmentation[2]. Among them, foam aluminum is highly potential as an electrochemical substrate material due to its light weight, low cost and high abundance[1][3]. The material has a very low density, good electrical conductivity, and resistance to acid and alkali corrosion[4]. In addition, aluminum foam's unique multi-space structure not only helps load more functional bacteria in the early stage but also increases the anode conducti on electron efficiency of SMFC with its excellent specific surface area.

Due to the good corrosion resistance, electricity conductivity, and high specific surface area, aluminum foam was chosen as the anode material.

Some references are as follows:

[1] Chen, J., Wang, Y., Li, S., Chen, H., Qiao, X., Zhao, J., Ma, Y., Alshareef, H.N., (2023). Porous Metal Current Collectors for Alkali Metal Batteries. Advanced Science 10(1), 2205695. https://doi.org/https://doi.org/10.1002/advs.202205695.

[2] Ding, Y., Zhang, Q., Rui, K., Xu, F., Lin, H., Yan, Y., Li, H., Zhu, J., Huang, W., (2020). Ultrafast Microwave Activating Polarized Electron for Scalable Porous Al toward High-Energy-Density Batteries. Nano Letters 20(12), 8818-8824. https://doi.org/10.1021/acs.nanolett.0c03762.

[3] Lamiel, C., Hussain, I., Ma, X., Zhang, K., (2022). Properties, functions, and challenges: current collectors. Materials Today Chemistry 26, 101152. https://doi.org/https://doi.org/10.1016/j.mtchem.2022.101152.

[4] Rossi, S., Bizzotto, M., Deflorian, F., Fedel, M., (2019). Study of anodizing process on aluminium foam to improve the corrosion behavior. Surface and Interface Analysis 51(12), 1194-1206. https://doi.org/https://doi.org/10.1002/sia.6610.

2. **In the manuscript, the authors included a virtual model diagram of the SMFC. Please include a realistic photograph of the setup in the subsequent revision to enhance clarity and provide a more comprehensive understanding.**

Response: Thanks for the suggestion. We will add a realistic photograph of the SMFC device to the supplementary material. The picture is as follows:

The left part of the picture is the SMFC photo, and the right part is the model groups. A plastic box (140.0×85.0×165.0 mm) was used as the SMFC reactor, with 1.50 kg soil and overlying water of 3.0 cm to simulate the flooded state during rice planting. The cathode was floated on the water surface while the anode was buried (about 3.0 cm from the bottom). The cathode and anode were connected to a 2000 Ω resistor using titanium wire. The water level was kept constant by daily replenishment.

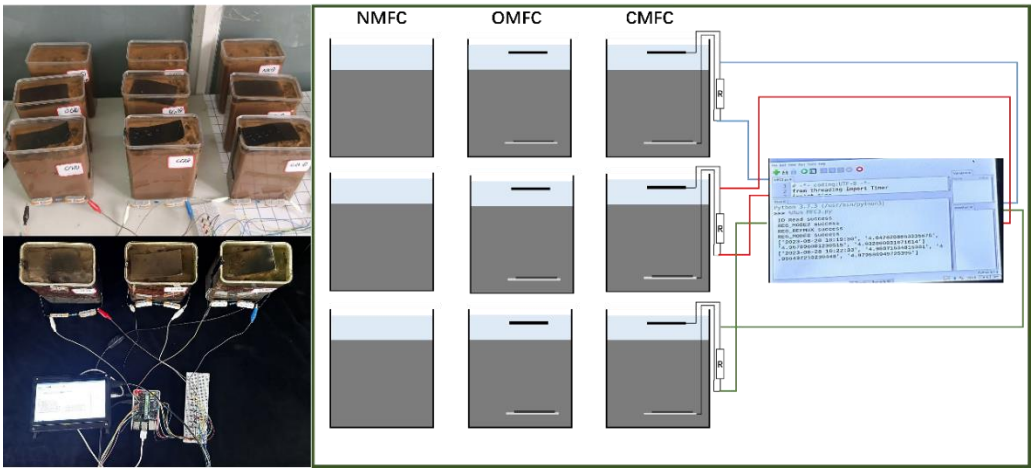

Fig. S1 SMFC structure and experimental grouping.

3. **In the abstract, the authors state: 'Fe_3O_4 nanoparticle as the cathodic catalyst effectively boosted power generation (0.97 V, 102.0 mW/m^2), whose porous structure and reducibility also contributed to Cr reduction and immobilization.' However, there is a lack of introduction regarding the cathode catalysts.**

Response: Thank you for your professional advice. In the revised manuscript, the related paragraph was revised as:

Graphite felt (GF) is fabricated from the polyacrylonitrile through a wiredrawing, high-temperature carbonization and graphitization process, in which a trapezoidal hexagonal ring-like lamellar structure is formed in the microfibrillar of the polyacrylonitrile-based carbon fiber. GF is widely used as a cathode due to its non-toxicity, stability, good electrical conductivity, chemical resistance. Moreover, it has a large three-dimensional active surface and mechanical integrity [1][2].

Some of the newly added references are as follows:

[1] Miao, J., Zhu, H., Tang, Y., Chen, Y., Wan, P., (2014). Graphite felt electrochemically modified in H2SO4 solution used as a cathode to produce H2O2 for pre-oxidation of drinking water. Chemical Engineering Journal 250, 312-318.

[2] Zhao, K., Quan, X., Chen, S., Yu, H., Zhang, Y., Zhao, H., (2018). Enhanced electro-Fenton performance by fluorine-doped porous carbon for removal of organic pollutants in wastewater. Chemical Engineering Journal 354, 606-615.

4. **The manuscript mentions the source and size of anode and cathode materials in section 2.2.2 Electrodes Preparation. However, some essential characteristics, such as the porosity and bulk density of the materials, are missing. Including these details is necessary to provide a comprehensive understanding of the electrode properties.**

Response: Thank you for your professional advice. In the revised manuscript, the related paragraph was revised as:

Aluminum foam (66.0 × 54.0 × 5.0 mm, porosity 60-80%, bulk density 0.50-1.10 g/cm^3) (SANZHENG Metal material, Chengdu, China) was used as anode. The anode microflora was derived from municipal sludge (Chengdu Sixth Sewage Treatment Plant, China) after acclimating with 100 mg/L Cr(VI). Before assembling, the aluminum foam was cultivated in the anode microflora for 2 weeks. Then the anode was tied to titanium mesh tightly with titanium wire. Graphite felt (GF) (100.0 × 50.0 × 3.0 mm, bulk density 0.10-0.15 g/cm^3) was used as the cathode (Table S1). Before use, it was cleaned, dried, and loaded with Fe_3O_4 as the ORR catalyst, as detailed in section 1 of the supplementary material. For characterization, we utilized a scanning electron microscope (SEM) to examine the structure and morphology of the electrode surface. In addition, we performed X-ray photoelectron spectroscopy (XPS) and energy dispersive spectroscopy (EDS) to analyze the valence state and element composition. The phase composition was determined using an X-ray diffractometer (XRD).

5. **Lines 138-143: Please provide detailed information on HRGs determination, including the reagents, instruments, and procedures used.**

Response: Thank you for your professional advice. In the revised manuscript, the related paragraph was revised as:

Total bacterial DNA was extracted using an E.Z.N.A.® Soil DNA Kit (Omega Biotek Inc., USA) according to the manufacturer's protocol. Specifically, 0.50 g sample, 0.50 g magnetic beads, and 1.0 ml SLX-Mlus Buffer were added in a 2.0 ml Eppendorf tube, and ground for 250 s under 45 HZ. Then added and mixed with 100 µl DS Buffer, and cultivated under 70 ℃ for 10 min and then 90℃ for 2 min. Then the mixture was centrifuged at 10000 g for 5 min under room temperature. 800 µl supernatant was moved to a new tube and added with 270 µl P2 buffer and 100 µl HTR reagent, and then cultivated under -20 ℃ for 5 min and then centrifuged again at 10000 g for 5 min. The supernatant was then moved to a new 2 ml tube and added with the same amount of XP5 buffer and mixed upside down for 8 min. After magnetic rack adsorption, discard the residual liquid, remove the tube, add 500 µL XP5 Buffer, and mix well. Then adsorbed again with a magnetic rack, discard the residual liquid, remove the tube, add 500 µL PHB, and mix well. Then adsorbed again with a magnetic rack, discard the residual liquid, remove the tube, add 500 µL SPW Wash Buffer, and mix well (repeat this step twice). Then the mixture was adsorbed again with a magnetic rack, discard the residual liquid, centrifuge the tube under 10000 g for 10 s. Then the beads were adsorbed again with a magnetic rack, discard the residual liquid, and let stand for 8 min. After that, the beads were added with 100 µL elution buffer, mixed, and let stand for 5 min. Finally, after adsorbing with a magnetic rack, the supernatant was moved to a new 1.0 ml Eppendorf tube, and total DNA was obtained for further use. The PCR reaction system was constructed. The abundance of HRGs in the surface soil of SMFC and OMFC anode after operation was analyzed using an SYBR Green real-time fluorescence quantitative PCR system (7500, Thermo Fisher, USA) (Wang et al., 2023a). The

soil of OMFC was used for comparison. The detected genes included HRGs (*chrA*, *chrB*, *chrR*, *recG*, *nfsA*, *zupT*, *fpvA*) and MGEs (*intI*, *tnpA02*, *tnpA04*, *tnpA05*). The primer sequences are provided in Table S2.

Technical corrections

1. **Please check typos throughout the text. As an example, supplementary material Table S5, ‘conFig.urations’ ?**

Response: Thank you for your careful examination. During revision, we will carefully review and revise the typos to ensure its accuracy.

The "conFig.urations " will be amended to "configurations ".

2. **Some figures in the manuscript need to be improved.**

Line 167: The annotations in Figure 1 are too small to be clearly seen.

Line 172: the icon in Figure 2 is blurred, and the the resolution need to be improved.

Response: Thank you for your kind suggestion. We will attach clear and eye-catching ICONS to the relevant images in the revised manuscript.

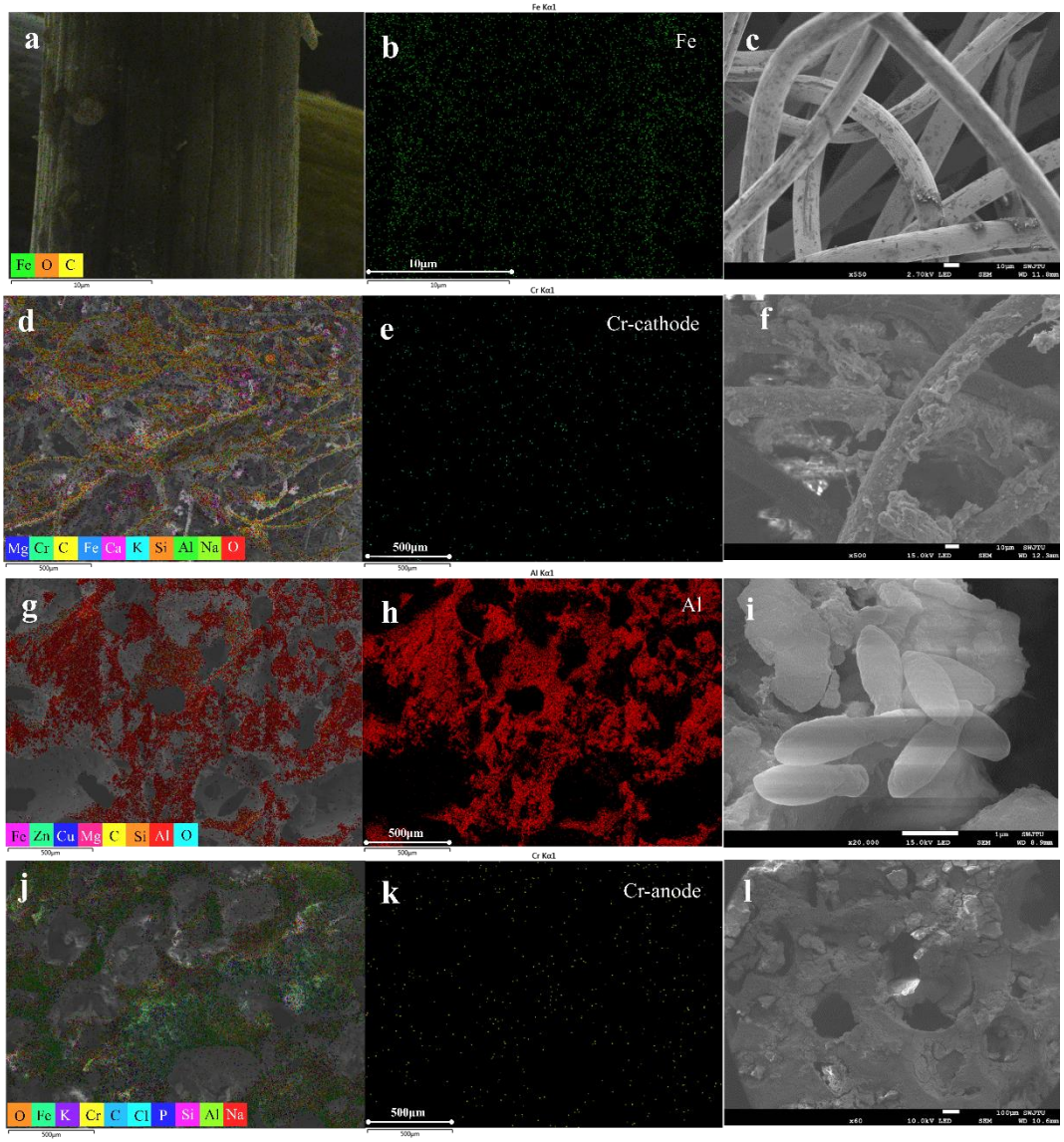

Fig. 1 Characterization of electrode materials before and after operation by EDS and SEM. (a-c) EDS and SEM images of cathode loaded with Fe_3O_4; (d-f) EDS and SEM images of cathode after the SMFC operation; (g-h) EDS and SEM images of anode microorganisms; (j-l) EDS and SEM images of the anode after SMFC operation.

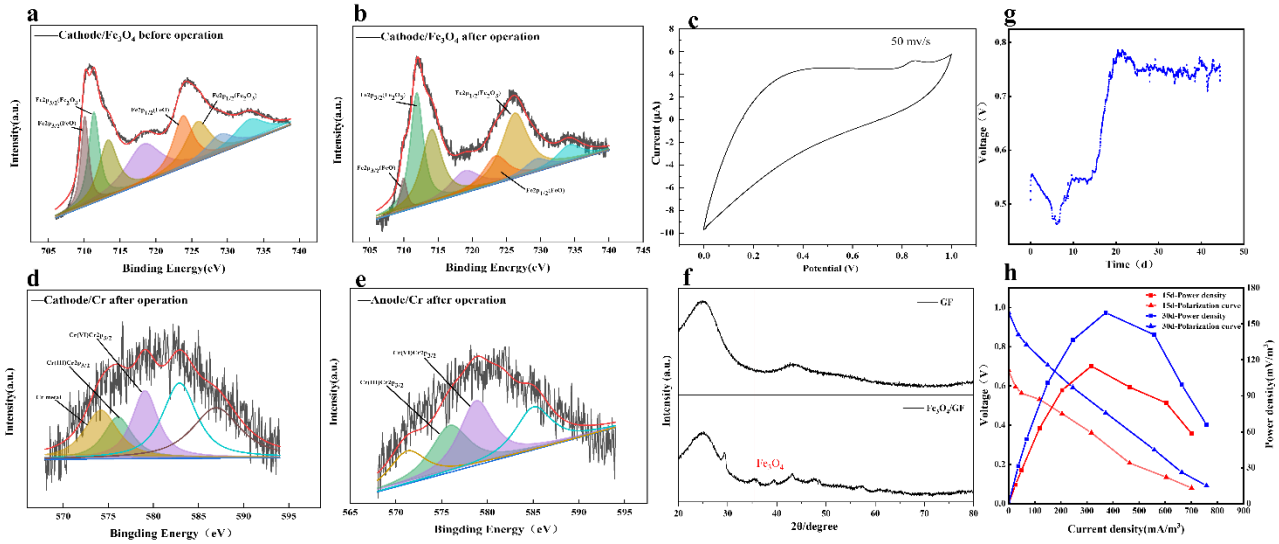

Fig. 2 Characterization of electrode materials. (a-b) Fe2p spectra of cathode/Fe_3O_4 composite cathode, (c) cyclic voltammetry (CV) curve of cathode/Fe_3O_4, (d-e) Cr2p spectra of GF composite cathode and Anodic Aluminum foam after operation, (f) XRD spectrum of the cathode-Fe_3O_4, Power generation performance of SMFC during long-term operation. (g)output voltage distribution, (h) polarization curves and power density curves (15-day vs. 30-day).