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.

The manuscript entitled " Cr(VI) reduction, electricity production, and microbial resistance variation in paddy soil under microbial fuel cell operation " has been written well and supported with sufficient experimental data. The authors found that during SMFC operation, soil physicochemical properties, enzyme activities, resistance genes, and microbial community structure closely interacted with each other. Although, the overall design of experiment and manuscript are fine, but some changes are required to be made in manuscript. Therefore, following changes should be included before publication:

We would like to thank the reviewers for their time and feedback on this manuscript. Please find our point-to-point responses below.

1. **Whether the SMFC has application prospects in practice, whether it can be applied to other soils except rice field soil, and whether the Fe_3O_4 will cause secondary pollution to the environment. Its advantage as cathode catalyst should also be mentioned. Please give a brief description.**

Response: Thanks for the suggestion.

This study presents an innovative treatment strategy that is economical and eco-friendly compared with conventional physicochemical methods. The soil MFC (SMFC) configuration was designed for in situ treatment and allows the anode to utilize the organic contents in sediment without a separator to divide the anode and cathode. Moreover, nutrients are passively supplemented during sedimentation from contaminated water to provide sustainable electron donors for SMFCs. The method can be used for in-situ contaminant treatment in a variety of environments, such as aquaculture ponds, inland lakes, and wetlands.

The iron oxides (Fe_3O_4/Fe_2O_3) are most commonly used as the modifier. The iron oxides modification mainly improves the kinetics activity of the reaction[1]. Iron-based materials due to the formation of dense flocs with good settling properties that remove phosphorus and other pollutants by adsorption and sweeping, have been widely used in environmental treatment[3]. Iron is a critical component of cytochrome C and iron-sulfur proteins, both of which are required by most electricigens and play a crucial role in the respiratory chain of microorganisms [2]. Therefore, the appropriate amount of Fe_3O_4 residue in the soil will not cause secondary pollution.

Some references are as follows :

[1] Li, J., Gao, H., (2008). A Renewable Potentiometric Immunosensor Based on Fe3O4 Nanoparticles Immobilized Anti-IgG. Electroanalysis 20(8), 881-887. https://doi.org/https://doi.org/10.1002/elan.200704094.

[2] Yu, B., Li, Y., Feng, L., (2019). Enhancing the performance of soil microbial fuel cells by using a bentonite-Fe and Fe3O4 modified anode. Journal of Hazardous Materials 377, 70-77. https://doi.org/https://doi.org/10.1016/j.jhazmat.2019.05.052.

[3] Venkateswarlu, S., Yoon, M., Kim, M.J., (2022). An environmentally benign synthesis of Fe3O4 nanoparticles to Fe3O4 nanoclusters: Rapid separation and removal of Hg(II) from an aqueous medium. Chemosphere 286, 131673. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.131673

2. Some of the formatting of the manuscript needs to be improved for ease of reading. Iines 278-290, the paragraph is too long to grasp the point and requires the author to break it up in appropriate places.

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

At the genus level (Fig. 4c), MFC operation presented a selection effect, with *Terrisporobacter* increasing from 0.81% to 13.71% and *Bacteroides* decreasing from 12.48% to 0.53% in CMFC anode. Compared with the Raw-A, many EABs in CMFC-A decreased, including *Clostridium_sensu_ stricto_5* (from 12.99% to 0.052%), *Clostridium_sensu_stricto_15* (from 4.70% to 0.47%), and *Enterococcus* (from 17.26% to 0.03%) (Choi, 2022; Zhang et al., 2023).

However, the *Desulfotomaculum* in CMFC-A increased to 3.32% compared with 0.003% in the soil (CMFC-S). Besides, soil indigenous bacteria including *Ramlibacter, Methyloversatilis*, and *Acinetobacter* colonized in the anode and elevated by 4.89~1 579 fold compared with soil. Nevertheless, multiple dominant genera in the soils decreased in CMFC-A than in OMFC-A. For example, *SBR1031*, *Bacteroidetes_vadinHA17*, and *Anaerolinea* were significantly increased in soil, but less in CMFC-A and OMFC-A. The electric field action to a certain extent helped the anode to resist external microbial intrusion to ensure the stability of the anodic microbial community.

3. Please explain the sampling process during the SMFC operation and if it will affect the normal operation and cause large errors?

Response: Thank you for your professional advice.

We used a plastic cylindrical straw with a diameter of 0.4 cm and a length of 16 cm as the sediment sampler. The SMFC sediment part is inserted by the sampler vertically at a specific time, and then quickly removed, and the upper, middle and lower parts of the sampler are mixed as a determination sample. And the fresh sample each time is only 8-16 g, only 0.2-0.4% of the SMFC. The total sampling amount shall not exceed 5% of the total population. Because the sampler is much smaller than SMFC, the disturbance is avoided to a great extent and the normal operation of SMFC is guaranteed.

4. Don't use the notion like 'we' or 'our' etc., as these are the redundant words (not the research words) for the standard journal manuscripts.

Response: Thank you for your kind advice. According to the reviewer's suggestion, the manuscript was carefully revised to make sure the use of research words, and some unnecessary redundant words were removed.

5. In part 2.5.3 HRG Fluctuation, does the author refer to extracted DNA for HRG detection? Please add the specific steps of extraction.

Response: Thank you for your kind advice. According to the reviewer's suggestion, the details of DNA extraction from the sample were added and marked in red in the material and method section. The detailed DNA extraction procedure was as follows:

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.

6. For gene extraction was it dried or fresh samples?

Response: Thank you for your careful work. For DNA extraction, 0.50 g fresh sample was used after homogenization. The detailed information was added and marked in red in the material and method section of 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 fresh 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.

7. Lines 157-159, " After operation, the typical peaks of Cr(Ⅲ), Cr(Ⅵ), and element Cr (576.1 and 578.92) were observed on both electrodes by XPS (Fig. 2d-e) " are insufficient to explain that the reduction and immobilization of Cr(VI) by the electrodes.

Response: Thank you for your careful work.

Chromium exists mainly in the III and VI oxidation states in soil. The peaks at 576.9eV and 579eV are typical peaks of Cr(Ⅲ) and Cr(Ⅵ), respectively. After operation, the typical peaks of

Cr(Ⅲ) and Cr(Ⅵ) can be observed on the X-ray energy spectra of both cathode and anode, and Cr(Ⅵ) and Cr(Ⅲ) are present on the cathode and cathode of SMFC. The results showed that at least the valence state of Cr changed on the electrode and Cr(VI) was reduced and fixed by the electrode[1].

Some references are as follows:

[1] Kim, C., Lee, C.R., Song, Y.E., Heo, J., Choi, S.M., Lim, D.-H., Cho, J., Park, C., Jang, M., Kim, J.R., (2017). Hexavalent chromium as a cathodic electron acceptor in a bipolar membrane microbial fuel cell with the simultaneous treatment of electroplating wastewater. Chemical Engineering Journal 328, 703-707. https://doi.org/https://doi.org/10.1016/j.cej.2017.07.077.