Supplementary Materials

Effect of wetland plant fermentation broth on nitrogen removal and bioenergy generation in constructed wetland-microbial fuel cells

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Summary

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Figure:

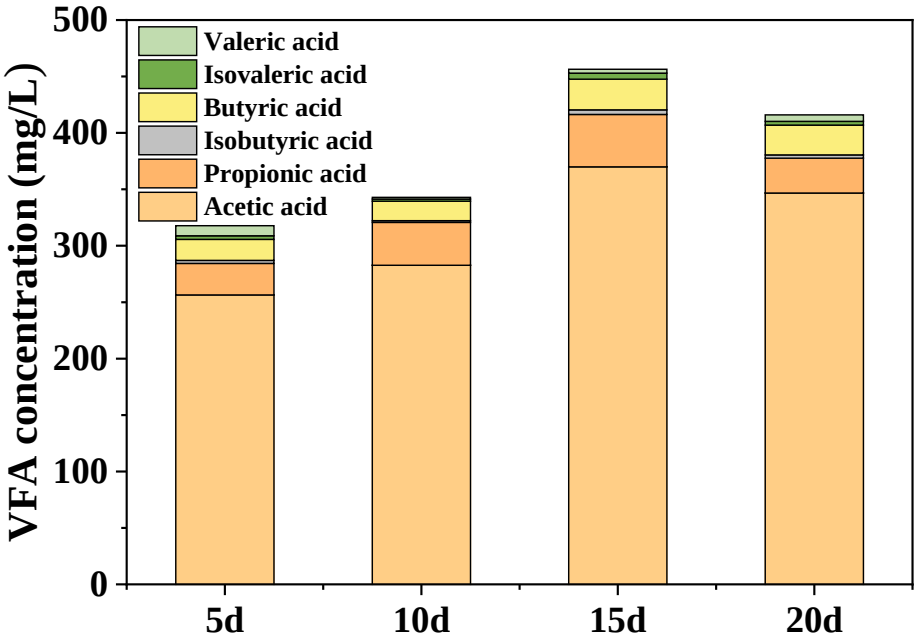

Fig. S1. VFAs concentration of fermentation broths.

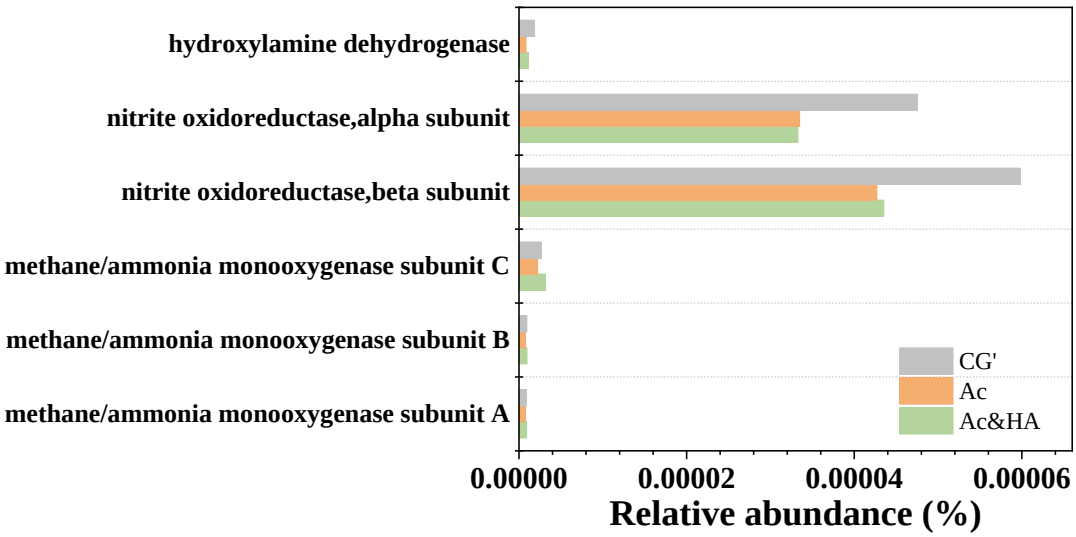

Fig. S2. Functional genes abundances related to nitrification were predicted with PICRUSt2.

Text S1: DNA extraction and Illumina MiSeq sequencing

Total DNA was extracted from the microbial sample from each treatment using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.), according to the manufacturer's protocol. All DNA samples were quality checked and the concentration was quantified by NanoDrop 2000 spectrophotometers (Thermo Fisher Scientific, Wilmington, DE, USA). Bacterial 16S rRNA gene fragments (V4 high variation zone) were amplified from the extracted DNA using primers 515FmodF (5'-GTGYCAGCMGGCGCGCGGTAA-3') and 806RmodR (5'-GGACTACNGGGTWTAT - 3'), and the following PCR conditions: 30 s at 95 °C, 30 s at 53 °C, and 45 s at 72 °C for 29 cycles. PCRs were performed with 4 μL 5 × TransStart FastPfu buffer, 2 μL 2.5 mM deoxynucleoside triphosphates (dNTPs), 0.8 μL of each primer (5 μM), 0.4 μL TransStart FastPfu DNA Polymerase, 10 ng of extracted DNA, and finally using ddH2O to make up 20 μL. Agarose gel electrophoresis was performed to verify the size of amplicons. Amplicons were subjected to paired-end sequencing on the Illumina MiSeq sequencing platform using PE300 chemical at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Amplicon sequence processing and analysis

After demultiplexing, the resulting sequences were merged with FLASH (v1.2.11) (Magoc and Salzberg, 2011) and quality filtered with fastp (0.19.6) (Chen et al., 2018). Then the high-quality sequences were de-noised using DADA2 (Callahan et al., 2016) plugin in the Qiime2 (Bolyen et al., 2019) (version 2020.2) pipeline with recommended parameters, which obtains single nucleotide resolution based on error profiles within samples. DADA2 denoised sequences are usually called amplicon sequence variants (ASVs). To minimize the effects of sequencing depth on alpha and beta diversity measure, the number of sequence from each sample was rarefied to 4000, which still yielded an average Good's coverage of 97.90%. Taxonomic assignment of ASVs was performed using the Naive bayes consensus

taxonomy classifier implemented in Qiime2 and the SILVA 16S rRNA database (v138).

References

1. Bolyen, E., Rideout, J.R., Dillon, M.R., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Asnicar, F., Bai, Y., Bisanz, J.E., Brejnrod, A., Brislawn, C.J., Brown, T.C., Caraballo-Roíguez, A.M., Chase, J., Cope, E.K., Silva, d.R., Diener, C., Dorrestein, P.C., Douglas, G.M., Duvallet, C., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibson, D.L., Gonzalez, A., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., Hooft, v.d.J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., Hippel, v.M., Walters, W., Wan, Y., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature biotechnology*, **37**(8), 852-857.
2. Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes,

S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods*, **13**(7), 581-583.

3. Chen, S., Zhou, Y., Chen, Y., Gu, J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, **34**(17), i884-i890.