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Dynamics of Microbial Communities Dominated by Putative Electrotrophic *Gammaproteobacteria* in Pilot-Scale Bioreactors Treating Mn(II)-Rich Mine Drainage

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Manganese (Mn) is one of the most abundant elements in the Earth's crust. In natural environment, Mn(II) is oxidized to insoluble Mn(III, IV) oxides by diverse bacteria under neutral pH conditions. Therefore, researches have demonstrated that the bacterial Mn(II) oxidation serves as promising tool for remediation of Mn-rich mine drainage. Recently, our research group established the pilot-scale bioreactors using Mn(II) oxidizing bacteria to treat drainages with 20 to 80 mg/L Mn(II) at an abandoned mining site in Japan [1,2]. Furthermore, our shotgun metagenomic analysis of the microbial communities suggested that putative chemolithoautotrophic bacteria possessing an extracellular electron transfer (EET) activity dominate in the bioreactor [1]. The previous study also suggested the involvement of diverse bacteria with MoxA-type Mn(II) oxidase enzymes in manganese oxidation. In this study, we aimed to show temporal dynamics of such putative electrotrophic bacteria and MoxA-like proteins-possessing bacteria because of the possible involvement in the Mn(II) oxidation in bioreactors. As results, the phylogenic analysis showed that the putative electrotrophs fall into a distinct clade within the class *Gammaproteobacteria.* Shotgun metagenomic analysis confirmed the abundance of genes encoding UetJ, a key protein in EET of Uet module, and MoxA-type protein in the bioreactor microbial community. Quantitative PCR using new primer sets targeting *uetJ*- and *moxA*-like genes, combined with 16S rRNA gene amplicon sequencing revealed the temporal dynamics of the gene copy numbers during the bioreactor operation. The reactor configuration and operational conditions affected largely the abundance of these genes. The results provide new insights into the Mn(II) oxidation driven by autotrophic bacterial communities and suggest that such communities play significant role in the Mn(II)-rich mine drainage bioremediation systems.

[1] Semoto et al., Performance of pilot-scale passive treatment tests of a contact oxidation manganese-oxidizing bacteria for manganese-containing mine water (2025), Proc. of IMWA2025, pp 874-879

[2] Watanabe et al., Accelerated manganese(II) removal by in situ mine drainage treatment system without organic subtrate amendment: metgaenomic insights into chemolithosutotrophic manganese oxidation via extracellular electron transfer (2024), *J. Environ. Chem. Eng.*, 12, 113314.