Abstract of Dissertation

論文の要旨

Title Strategies for enrichment of manganese-oxidizing bacteria to enhance removal of azo dye through biological Mn-redox processes

(Mn 酸化還元を利用したアゾ染料排水の脱色促進のためのマンガン酸化細菌の集積戦略)

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Manganese-oxidizing bacteria (MnOB) that are capable of oxidizing Mn(II) to biogenic manganese oxides (bio-MnOx), are ubiquitous in nature and have attracted attention in water and wastewater treatment due to their biotechnological potential of microorganisms for removal organic contaminant, and the high adsorption and oxidation ability of the generated bio-MnOx towards inorganic and organic pollutants. In this study, several strategies to obtain favorable conditions for MnOB enrichment in a continuous down-flow hanging sponge (DHS) reactor were studied, including the preferred organic substrates for supporting MnOB growth and the effect of residual Mn(II) on the Mn(II) oxidation performance. The potential of the enriched MnOB and bio-MnOx for the removal of recalcitrant azo dye compounds were also investigated.

Three different organic substrates; K-medium, methanol, and sterilized activated sludge (SAS), can be used to support MnOB enrichment in a continuous DHS reactor. Methanol-fed reactor exhibited the highest Mn(II) oxidation performance of 0.49 kg $Mn \cdot m^{-3} \cdot d^{-1}$. The microbial community of the reactor was strongly governed by organic substrate, and *Comamonas*, *Pseudomonas*, *Mycobacterium*, *Nocardia*, and *Hyphomicrobium* were found to play an important role in Mn(II) oxidation in the reactors. MnOB enrichment can be accelerated by employing abiotic-MnO_2 in the sponge media. The higher residual Mn(II) in the reactor was found to inhibit the Mn(II) oxidation performance. The relative abundance of putative MnOB, *Hyphomicrobium*, was inhibited by the higher residual Mn(II). Maintaining residual Mn(II) at low level is a key factor to achieve sustainable high Mn(II) oxidation performance of the reactor.

The potential of enriched MnOB and bio-MnO_x for the removal of azo dye Acid Orange 7 (AO7) was evaluated. Even though the reactor achieved high Mn(II) oxidation rate, it was unable to remove AO7. The bacterial isolates from the reactor were found to possess Mn(II) oxidation ability but not AO7 removal. There was no catalytic effect of Mn(II) oxidation on the decolorization of AO7. In addition, sodium acetate, in term of COD, could be effectively removed in the Mn(II)-oxidizing reactor. A sulfonated azo dye compound, Bordeaux S (BS), was also resistant to biological Mn(II) oxidation process under aerobic conditions, but it was easily decolorized under anaerobic conditions. Anaerobic decolorization of azo dye was strongly dependent on the co-substrate addition that acts as the electron donor for reductively cleavage of azo bond. Interestingly, this study showed that the abiotic-MnO_2-containing reactor achieved higher decolorization efficiency that the reactor devoid of abiotic-MnO_2 at a low K-medium addition. The presence of abiotic-MnO_2 was beneficial not only to decompose extracellular polymeric substances (EPS) to more utilizable substrates, which can serve as an electron donor at organic source deficient, but also as a conductive material that promotes electron transfer. Unfortunately, anaerobic decolorization was unable to completely degrade azo dye, and its intermediate products remained in the effluent as indicated by a high COD concentration.

Integrated anaerobic-aerobic DHS reactor under manganese redox conditions was applied to further degrade azo dye wastewater. The results showed that only small portions of

COD (< 30%) can be removed in the aerobic reactor, and the residual COD corresponded to the initial BS concentration, which suggested that intermediate products of BS were resistant to both anaerobic and aerobic treatment. Interestingly, nitrogen loss was found to occur in both reactors. In the anaerobic reactor, ammonium, a transformation product of co-substrate K-medium, was oxidized to nitrogen gas by manganese oxides. In addition, sulfate reduction may also contribute to the anaerobic ammonium oxidation. In the aerobic reactor, nitrification occurred on the sponge surfaces, and denitrification was possible to occur inside the sponges by coupled with manganese oxides reduction, as indicated by the higher Mn(II) in the effluent. Thus, the Mn oxides-containing reactor would be a promising method for the treatment of high nitrogen-containing wastewater.

This current study extends our knowledge on the enrichment strategies of MnOB and their potential for removal organic pollutants. The abiotic MnO_2-containing reactor has potential applications in the treatment of high Mn-containing wastewater from various industries, such as mine drainage, hydrometallurgical processing, steel and alloy industry. In addition, the Mn oxides-containing reactor would be a promising method for the treatment of high organic nitrogen-containing wastewaters, such as municipal wastewater, food industries, etc. Further studies are required to clarify the role of manganese oxide in regulating ammonium removal.

Keywords: Manganese-oxidizing bacteria, azo dye decolorization, Mn redox, manganese oxide, nitrogen removal